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THE JOURNAL OF PERIODONTOLOGY is published to serve as the professional journal of periodontology; to keep dentists in touch with research being carried on in this field and related sciences; to stimulate greater interest in the supporting tissues of the teeth and their relation to oral and general health.

The Journal is the official organ of the American Academy of Periodontology and will contain, therefore, papers of scientific and clinical interest which are presented at the annual meetings of that society, as well as articles submitted direct to the Editor. Obviously opinions expressed do not necessarily represent those of the Editor or the American Academy of Periodontology.

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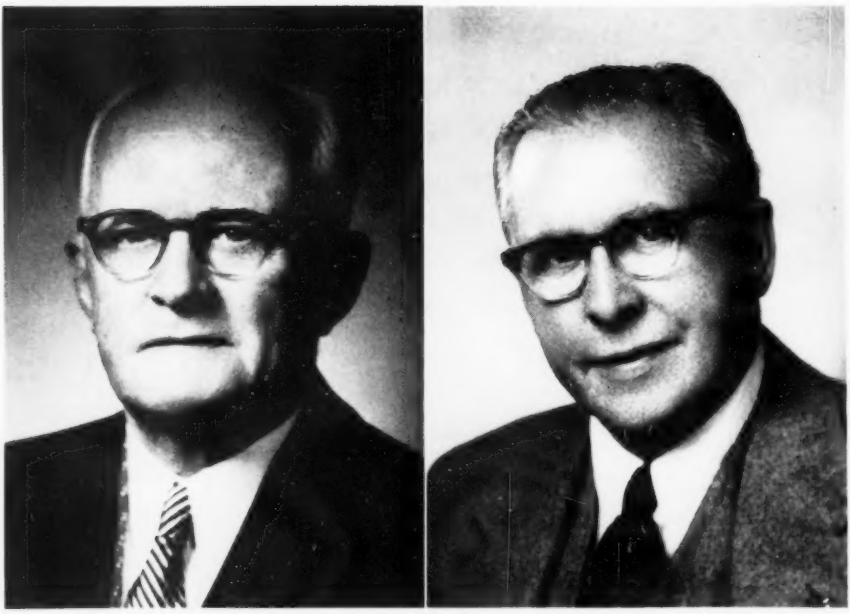

CARLOS H. SCHOTT, D.D.S. — EDWARD L. BALL, D.D.S.

Cincinnati, Ohio

Periodontology owes a debt of gratitude to two outstanding dentists in Cincinnati, Dr. Carlos H. Schott and Dr. Edward L. Ball. These two men have worked together many long years for the advancement of dentistry and periodontology.

Dr. Carlos H. Schott was born in Harrison, Ohio, June 8, 1882. He attended the Ohio College of Dental Surgery in Cincinnati, Ohio, graduating in 1903. He served as President of the Ohio State Dental Association and the American Academy of Periodontology, was also a Trustee of the American Dental Association for seven years and has been most active in organized dentistry.

Dr. Edward L. Ball was born in Indianapolis, Indiana, April 3, 1889 and was graduated from the Ohio College of Dental Surgery in 1919. He is a past President of the Ohio State Dental Association, American Academy of Periodontology and the Pierre Fauchard Academy. He has been very popular as an essayist on the subject of periodontology, and his presentations, backed up with his banjo, have made him one of the most popular speakers in this generation.

THE JOURNAL OF PERIODONTOLOGY

APRIL, NINETEEN HUNDRED SIXTY ONE

Post-Operative Care of the Infra-Bony Pocket

by GERALD M. KRAMER, D.M.D.,* AND J. DAVID KOHN, D.D.S.,** *Boston, Mass.*

THE surgical treatment of the infra-bony pocket has been described adequately by Goldman,[1] Prichard[2] and others. It is important to emphasize that the postoperative care of infra-bony pockets so treated deserves careful attention. The surgical procedure is carried out as follows:

1. A gingivectomy to the crest of the bone

2. Curettment of the granulation tissue from the bony walls of the pocket (later improvement—careful removal of the transeptal fibers which over-lie the defect area)

3. Debridement and planing of the root surface

4. Enucleation of the epithelial attachment

5. Scarification of the near-by epithelium

Upon completion of these steps, the wound is dressed in the following manner:

1. Adhesive foil or telfa is cut to shape and placed to cover that portion of the bony hollow where bony fill-in is anticipated

2. Surgical pack is placed over the positioned adhesive foil and the gingivectomy wound; great care is taken to prevent pack from entering the bony defect

3. The pack and the tinfoil are removed one week postoperatively

4. The operated site is then similarly redressed for an additional week or two until epithelization of the wound is adequate.

The goal of this procedure is to achieve a more coronally established attachment apparatus. Included within this goal is the concept of new bone development.

The question is whether or not bone is established appositionally or is differentiated from the clot. Until recently, most clinicians have based their therapy upon establishment and protection of the blood clot. However, based on Linghorne's[3] work on dog's teeth and Ham's[4] fracture experiments, one may assume

*Assistant Professor, Department of Stomatology, School of Medicine, Boston University.

**Instructor, Department of Stomatology, School of Medicine, Boston University.

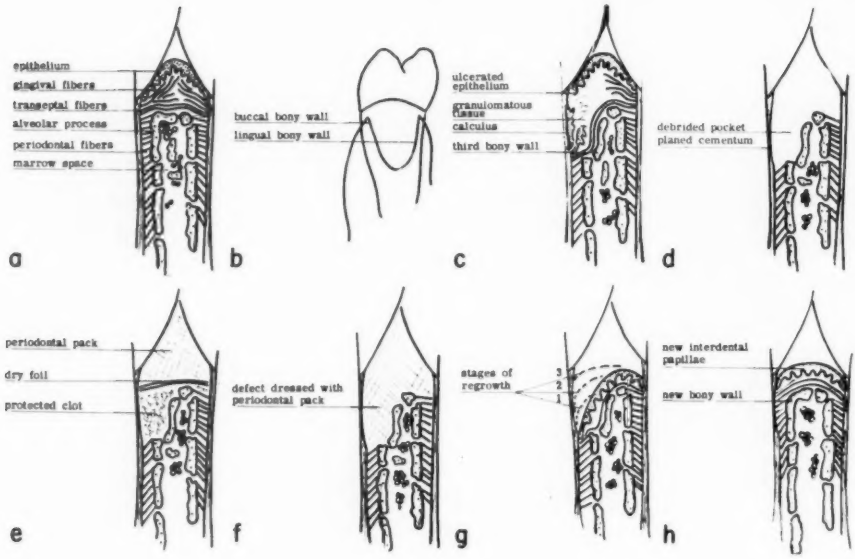

Fig. 1. A series of diagrams showing a typical interdental area in health, disease, with the two dressing techniques employed, and post-operatively. a. normal, b. periodontal pocket showing two of the three bony walls, c. periodontal pocket showing the third osseous wall, d. the lesion treated and ready to receive dressing material, e. original clot protection type of dressing, f. the defect dressed by our method, g. hypothetical repair and growth pattern, h. the end result showing new bony growth and attachment.

that the need for clot protection does not exist.

The implication in these works is that the clot does not seem to take part in new bony formation, but serves as a dressing which protects the wound. They have demonstrated that in repair experiments, bone develops specifically from osteogenic cells which are usually seen covering and lining bone surfaces. These may possibly be helped in their function by undifferentiated cells from the bone marrow. These works may offer a possible explanation for the success of those surgically treated infra-bony pockets in which the clot is lost or displaced inadvertently by pack.

Because we do get successes in these cases, and because of the manipulation difficulty in the clot protection procedure, we decided to try pack placement into the surgical defect as a routine procedure.

Our standard technique for packing the classical surgical infra-bony pocket procedure is as follows:

1. Wash the operated field thoroughly with warm water

2. Place soft pack gently to the base and sides of the bony wound

3. The remaining gingivectomy area is covered with pack and fused to the previously placed pack

4. Care is taken not to disturb this area for one week, after which, all of the pack is removed including that occupying the defect area

5. Topical anesthetic (Dyclone Creme, 1%) is applied to diminish pack-placement discomfort

6. A new soft pack is inserted in the same manner as the original pack

7. At the end of a two-week period, all packing is removed. If the gingivectomy

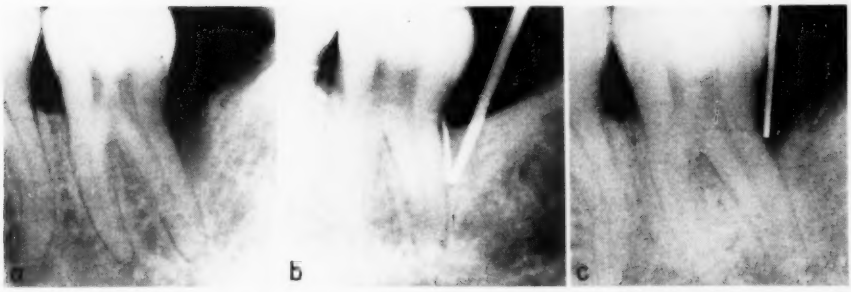

Fig. 2. a. mandibular second molar exhibiting a deep, distal, three-walled infra-bony pocket. b. soft gutta percha point used to demonstrate the extent of the defect. c. silver cone placed to position with force. This shows the attachment and bony fill-in obtained one year post-operatively. Pack was placed directly into the defect.

area is adequately healed, fresh pack is placed only in the deformity area. If, at this time, the gingivectomy area is not healed, it must also be packed. The fresh pack remains in position one to three more weeks with weekly repacking intervals. This is done until it is felt that proper epithelization has occurred. Between these intervals, the patient is allowed to clean around the packed defect area. Note that it is important to debride the area completely at packing intervals.

At the time of the last pack removal, the patient takes upon himself the responsibility of maintaining a healthy gingival situation in the following manner:

1. The day of the pack removal, frequent warm water rinses are begun

2. For the following two or three days, care is taken to avoid chewing on the operated side

3. Brushing with a soft brush begins within twenty-four hours

4. Interdental stimulation is started four to five days postoperatively

5. Sometimes the use of pipe cleaners is employed interproximally if the soft debris accumulated on the root surfaces cannot be removed in any other manner

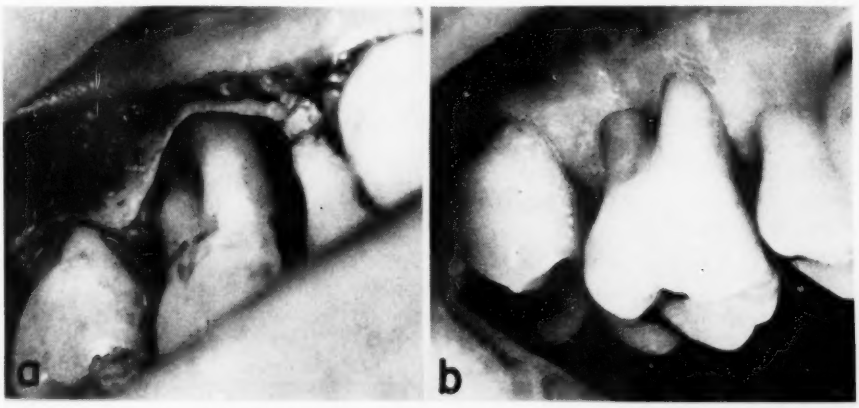

Fig. 3. a. Circumferential infra-bony pocket of the maxillary, molar region treated classically with the exception of pack placement. The pack was placed directly into the bony pocket. b. Same area seen eight months later.

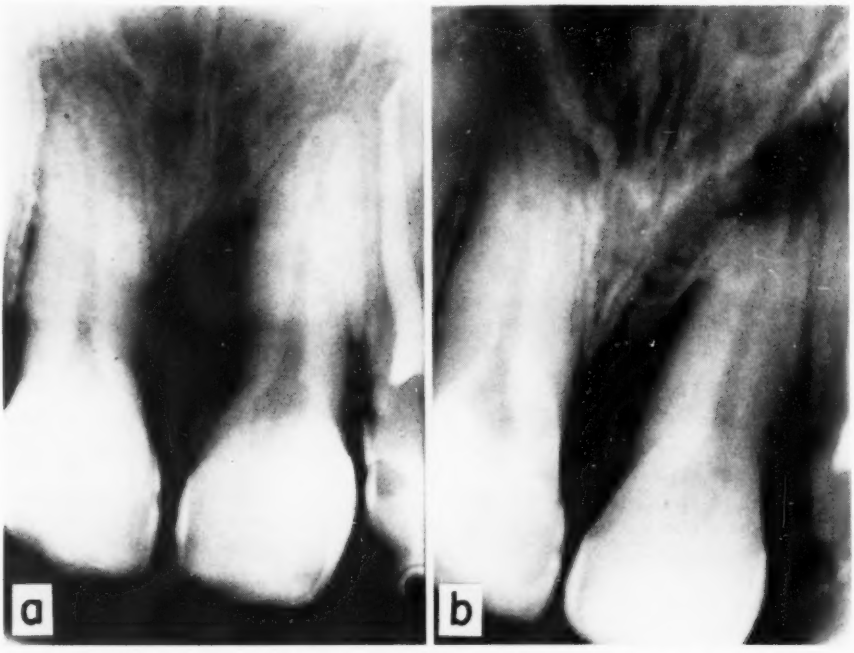

Fig. 4. a. Radiograph of alveolar bone loss seen on the mesial aspect of a maxillary central incisor. b. This area seen one year post-operatively. Pack has been placed directly into the defect area. Notice the unaided closure of the space between the two central incisors.

The time required to fill in the cup-like depression to the brim varies from a few weeks to a few months. During most of the filling-in period, the depressed area appears to be well epithelized. It takes on a healthy pink appearance. Subsequent plastic procedures to reshape the edges of the osseous defect are not needed. The tissue seems to assume what we consider to be a more desirable topography. This topography can be described as one in which the gingival margins are thin and closely adapted to the teeth. They exhibit minimum sulcus depth.

To this date no one has explained satisfactorily the exact mechanism of new bony growth in these infra-bony pockets. In trying to analyze the reasons for success in these cases, we realized that there are certain procedures we perform empirically which may be effectual. These are (1) the use of bacteriacidal and fungicidal agents in the pack, (2) occlusal adjustment and/or splinting, (3) selection of cases limited to three osseous-walled infra-bony pockets, (4) meticulous maintenance by the patient and by ourselves, and (5) our infra-bony operations being a part of a larger surgical procedure.

This paper introduces a method of dressing infra-bony pockets. We consider this method to be more facile and capable of producing the desired therapeutic result. On a predictable basis, this technique may or may not accomplish the percentage of success achieved in the clot protection method. However, the success met with this procedure gives credence to the possibility that clot protection is unnecessary.

9 Nahant Street
Lynn, Massachusetts

REFERENCES

1. Goldman, H. M., "A Rationale for the Treat-

ment of the Infra-bony Pocket," J. Periodont., 20 (1949), 83-92.

2. Prichard, John, "The Infra-bony Technique as a Predictable Procedure," J. Periodont., 28 (July 1957) 202-216.

3. Linghorne, W. J., and O'Connell, D. C., "Studies in the Regeneration and Reattachment of Supporting Structures of the Teeth: II. Regeneration of the Alveolar Process," J. D. R., 30 #5 (October 1951) 604-614.

4. Ham, A. W., and Harris, W. R., The Biochemistry and Physiology of Bone, Bourne, G. H. (New York c-1956) Academic Press, pp. 475-505.

An Evaluation of the Antimicrobial Effect of the Fluid Exudate from the Clinically Healthy Gingival Crevice

by A. A. COLLINS AND J. B. GAVIN, *Dunedin, New Zealand*

IT has recently been demonstrated by Brill and his co-investigators[1,2] that there is a passage of tissue fluid from the sub-epithelial tissues of the gingiva into the gingival crevice, and also that the amount of this exudate is substantially increased by mechanical stimulation[3,4] or vigorous chewing.[5]

The mechanical cleansing action of this outgoing stream of tissue fluid has been shown by the removal from the gingival crevice of introduced particulate matter[6,7] including bacteria.[6,8] Further, due to the presence in the fluid of amino acids[5] and plasma proteins,[3] Brill has suggested[3,5,6] that it may contain certain humoral defense factors which could have an antimicrobial action within the gingival crevice.

This study was undertaken to investigate the second of these suggested defense mechanisms.

MATERIAL AND METHODS

Fifteen male, third-year dental students aged between eighteen and twenty-five years were selected from a larger number for this study. In each case the subject was healthy and maintained a high standard of oral hygiene. No student was considered if any signs of deposits on the teeth or inflammatory changes involving the gingiva were clinically evident. The gingival crevices about incisor, canine, and premolar teeth of both arches were investigated.

Department of Basic Dental Sciences, Otago University Dental School, Dunedin, New Zealand.

Following the method of Brill and Krasse[1], the fluid was collected by means of sterile tapered filter paper strips. These were placed in the labial or buccal aspect of the carefully isolated and dried gingival crevices for three minutes. The strips of filter paper had been previously sterilised by autoclaving at approximately 120°C for ten minutes and were subsequently handled only with sterile metal dressing forceps. Any strips accidentally contaminated by saliva or by contact with the lips or cheeks were discarded.

To obtain maximum amounts of this fluid its flow was stimulated by each subject chewing a large volume (20 cc) of paraffin wax for ten minutes. This has been shown to increase the flow of this tissue fluid by about six times[5]. Following this stimulation samples were taken from four or five crevices from each subject.

As controls, samples were taken from two crevices from each subject prior to stimulation, and one strip was moistened with saliva in the vicinity of the crevices after stimulation.

It was intended to evaluate the antimicrobial effect of this fluid by placing the strips containing it on to 5% blood agar previously inoculated with various strains of oral bacteria and to observe during incubation any inhibition of bacterial growth about the strips. Samples and controls from the first five subjects were placed on media previously inoculated with a strain of Streptococcus salivarius. However, in the light of the results of these cultures samples and controls from the other ten subjects were placed on to uninoculated 5% blood agar. The blood agar plates were incubated overnight at 37°C.

RESULTS

In no case was there any inhibition of the uniform growth of Streptococcus salivarius in the vicinity of the strips. Also, in all cases a confluent growth of organisms was observed on and beneath those portions of the strips which had absorbed the crevicular exudate before or after stimulation. The remaining portion of the strips showed no growth. The control strips containing absorbed saliva showed a similar confluent growth.

Similar results were obtained from the samples and controls placed on uninoculated blood agar plates, the growth of organisms covering completely that area of the strip containing the absorbed exudate or saliva.

The thirty strips containing absorbed exudate from unstimulated crevices, the seventy-one strips taken after stimulation, and the fifteen strips containing absorbed saliva gave in every case a similar pattern of growth. The organisms present in each case were mixed and included Neisseria, Diphtheroids, Streptococci, Staphylococci and other common oral organisms.

DISCUSSION

The present findings indicate that antimicrobial factors, if present in the crevicular exudate, exert no demonstrable bacteriostatic or bactericidal effect under the conditions of this experiment. However, due to the derivation of this tissue fluid from the blood stream[1] and to the presence within it of plasma proteins[3] and amino acids,[5] it seems reasonable that it should contain at least some of the humoral defense factors found in other body fluids. These factors may be either specific or non-specific in action.

It is thought unlikely that many of the oral organisms would have previously entered the tissues and thus stimulated the production of specific antibodies. The profuse growth of organisms obtained in this study supports the view that specific antibodies, if present in the crevicular exudate, have little significant effect on the total flora of the gingival crevice.

Much has been written on the less specific humoral defense factors[9,10,11,12] but, as yet, their moment in tissue resistance to infection appears in doubt. Wilson and Miles[11] state that bactericidal and bacteriostatic substances can be isolated from mammalian tissues, many of them basic polypeptides and proteins, but their role in the tissue defenses is as yet problematical. Skarnes and Watson[12] suppose that these antimicrobial substances involved in natural resistance do not exist as such in normal tissues, but arise in response to physiologic changes which accompany stress. This latter view is in accordance with the present findings that fluid collected from the clinically healthy gingival crevice has little effect on the growth of oral organisms.

Other factors, also non-specific in action, are those of the opsonin type which, although having little bacteriostatic effect in themselves, render organisms more amenable to phagocytosis. In an inflammatory response by the gingivae to introduced particulate matter including bacteria[7,8] leukocytes are present, but in the healthy gingival crevice these mechanisms are thought to have little appreciable effect on the bacteria entering the crevice. Should organisms enter the underlying gingival tissues, however, prior contact with factors of this type could reduce their effective pathogenecity.

The profuse growth of organisms obtained in this study, and in subsequent investigation[13] has led the authors to question the evidence supporting the belief that the clinically healthy gingival crevice, free from calculus or deposits, is as a rule, sterile.[7,8]

SUMMARY

The tissue fluid exudate from the clinically healthy gingival crevice has no appreciable bacteriostatic or bactericidal effect.

This fluid did not inhibit the growth of many common oral bacteria, indeed it gave a profuse growth of mixed oral organisms casting some doubt on the opinion that the clinically healthy gingival crevice is sterile.

The authors wish to acknowledge the assistance and co-operation of Associate Professor F. R. Shroff, Head of the Department of Basic Dental Sciences at the University of Otago Dental School, during this investigation.—*P.O. Box 647, Dunedin, C.I., New Zealand.*

REFERENCES

1. Brill, N. and Krasse, B. The Passage of Tissue Fluid into the Clinically Healthy Gingival Pocket. Acta Odont. Scand. 16:233, 1958.

2. Brill, N. and Bjorn, H. Passage of Tissue Fluid into Human Gingival Pockets. Acta Odont. Scand. 17:11, 1959.

3. Brill, N. Influence of Capillary Permeability on flow of Tissue Fluid into Gingival Pockets. Acta Odont. Scand. 17:23, 1959.

4. Brill, N. and Krasse, B. Effect of Mechanical Stimulation on flow of Tissue Fluid through the Gingival Pocket Epithelium. Acta Odont. Scand. 17:115, 1959.

5. Brill, N. Effect of chewing on flow of Tissue Fluid into Gingival Pockets. Acta Odont. Scand. 17:277, 1959.

6. Brill, N. Removal of particles and bacteria from Gingival Pockets by Tissue Fluid. Acta Odont. Scand. 17:431, 1959.

7. Waerhaug, J. The Gingival Pocket. Odont. Tdskr. 60, Suppl. 1., 1952.

8. Waerhaug, J. and Steen, E. The presence or absence of bacteria in Gingival Pockets and the reaction in healthy pockets to certain pure cultures. Odont. Tdskr. 60:1, 1952.

9. Appleton, J. L. T. Bacterial infection with special reference to Dental Practice. 4th edn. chap. 13, Lea and Febiger, 1950.

10. Dubos, R. Bacterial and Mycotic Infections of man. 3rd edn. Chap. 4, Lippincott, 1958.

11. Wilson, G. S. and Watson, D. W. Topley and Wilson's Principles of Bacteriology and Immunity. 4th edn. chap. 52, Williams and Wilkins, 1955.

12. Skarnes, R. C. and Watson, D. W. Antimicrobial factors of normal tissues and fluids. Bact. Review, 21:273, 1957.

13. Gavin, J. B. and Collins, A. A. The Occurrence of Bacteria in the Clinically Healthy Gingival Crevice. To be published.

Book Review . . . .

THE PRACTICE OF PERIODONTIA

The Practice of Periodontia, Sidney Sorrin, Editor, First Edition, 496 pages. McGraw-Hill Company, Incorporated. New York, New York, 1960. $19.50.

The Practice of Periodontia is the latest edition to many texts on periodontia available to the dental profession. The book apparently is written to include a broad group of readers from the undergraduate student to the specialist.

The basic histology, physiology, and pathology are discussed in the introductory chapters, followed by etiology, diagnosis, and prognosis. The section on occlusal disharmonies is carefully explained and well illustrated. Considerable emphasis is placed on the role of occlusion as an etiologic and treatment factor in periodontal disease.

Since minor tooth movement is being widely practiced by the periodontist, a chapter with many drawings showing a multitude of effective procedures in this field is included.

Treatment planning and the various accepted forms of treatment are discussed in detail. The book also has an exhaustive index which in itself is a reflection of the thoroughness of the authors.

While this text does not necessarily contribute anything new that is not available in other texts, it is a well-written book and covers the field of periodontia adequately.

Henry M. Swenson, D.D.S.

The Dynamics of a Tooth in Function

by GILBERT J. PARFITT, F.D.S.; M.R.C.S., L.R.C.P.; D.M.D., *Birmingham, Alabama*

TEETH that are clinically firm possess a measurable degree of labial mobility which varies with the time of day, with exercise, and with systemic disorders.[1] The degree of lateral force deliberately applied in tooth mobility measurements (500 gm.) occurs naturally many times during the day and night in the clenching of teeth, in mastication of food, in swallowing, and during speech. Although considerable force is exerted by the muscles of mastication[2] and by the lips and tongue,[3] muscular forces are not generally concentrated on one tooth,[4] but are spread over many teeth, so that the amount of lateral force on a single tooth during a particular movement is not known. Measurements were, therefore, undertaken to find the degree of lateral movement of an individual tooth during the physiological functions of mastication, swallowing, and speech.

METHOD

Instruments that transform movement into electric current are commercially available. They are variable permeance transducers. These instruments consist of two coils through which floats a movable magnetic core (Fig. 1). The permeance of one coil increases and the other decreases as the core is moved. The two coils act as two legs of an electrical bridge circuit, the other two legs being in an external circuit provided by a translator unit. The translator unit supplies the transducer with an oscillating current (10 kc.) and amplifies and demodulates the signal produced by the difference in potential across the bridge. This apparatus has already been described.[5] The only adaptation required for the present measurements was to hold a transducer to the labial surface of the tooth without hindering speech or the mastication of food. A frame was made from an upper clutch of a Rower occlusolator, an instrument devised to record extraorally the borderline movements of the mandible. The clutch consists of two arms which pass between the lips and embrace the buccal surfaces of the upper dental arch as far forward as the canines. The arms are hinged and are brought together by a screw. A remarkably firm grip, particularly in the antero-posterior direction, was made to the teeth by means of acrylic impressions, resurfaced with compound impression wax attached to the arms of the clutch. A transducer was held by means of a lightweight universal clamp to the center of the extraoral hinge. These clamps are used in maxillofacial surgery in extraoral pin fixation. The entire assembly (Fig. 2) weighed only 50 grams and interfered

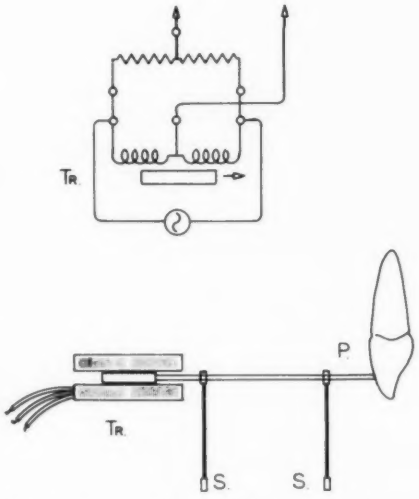

Fig. 1. Circuit diagram and section of transducer assembly. TR—Crescent Engineering and Research Type ZD subminiature rectilinear transducer, sensitivity 2.2 mv/v/.001", impedance 800 ohms. P—Probe. SS—Cantilever springs carrying probe and core.

This study was supported in the School of Dentistry, University of Alabama by Research Grant D-307 from the National Institutes of Health, United States Public Health Service.

very little with speech, and not at all with the mastication of food or swallowing. The incision of food, however, had to be performed very carefully in case an incisor tooth became buried in food, displacing the delicate probe attached to the core of the transducer bearing upon the labial surface of the tooth.

The lip pressure against the labial surface of the upper incisor tooth at rest was found to be 3-5 gm., and during function rose to 20 gm. The contact pressure of the transducer probe against the labial surface of the tooth was, therefore, adjusted to 5 gm., which replaced the pressure of the lip displaced by the instrument.

The movement of the tooth calibrated in hundredths and in thousandths of a millimeter was read on a meter, or an X-Y recorder to chart force and movement, and also on a time-movement recorder. The time recorder set at four inches-per-minute was used to separate the impulses caused by the movement of the tooth and to observe the speed of deflection and the return of the tooth to its original position. A continuous record of the tooth movement could, therefore, be made in the labio-lingual direction during any particular mandibular function.

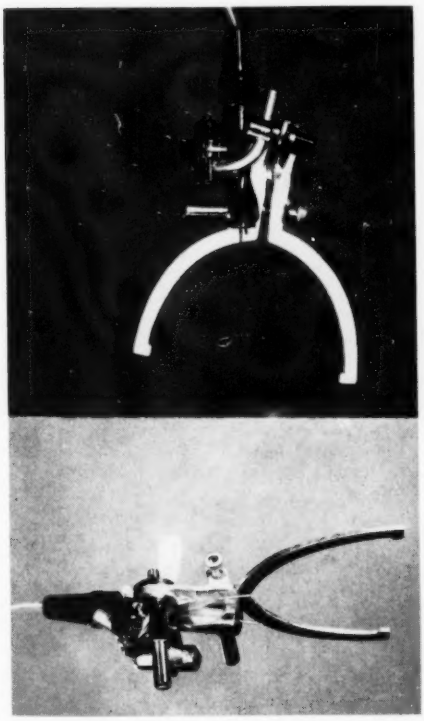

Fig. 2. Transducer in suspension housing, clamped to occlusalator clutch.

The tooth chosen for observation was a right upper central incisor which had never been the sight of periodontal disease. The gingival margin was not inflamed, and the crevice depth was less than 2 mm. There was no absorption of the 1.2 cm. root or bone recession, the bone level being 2 mm. from the cemento-enamel junction. The periodontal membrane space was even and approximately 0.2 mm. width all round the tooth. Clinically firm and giving a high note on percussion, the tooth showed, by Mühlemann's micrometer measurement technique, a labial movement of 0.04 mm. with 500 gm. force, with a contact pressure of 50 gm. on the buccal surface of the tooth, and 0.03 mm. lingual movement (Fig. 3). The total labio-lingual movement, therefore, was 0.07 mm., which is smaller than the normal range of movement reported by Mühlemann. (It was noted that a movement of 0.02 mm. could be appreciated by palpation.)

Confirmatory measurements made with the transducer showed a labial movement of 0.04 mm. with 500 gm. force and a contact pressure of 5 gm., and a total labio-lingual movement of 0.073 mm. with 500 gm. acting first in a labial then in a lingual direction. This particular incisor overlapped the lower incisors by more than two-thirds, and the long axis subtended an angle of 72.7° with the occlusal plane and 166.2° with the long axis of the lower incisor teeth occluding with it. Both upper and lower incisors were lingually inclined compared to the average. The incisors occluded in centric.

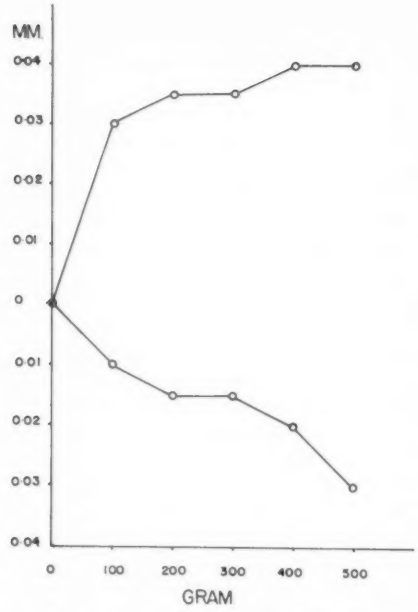

Fig. 3. Labial (up) and palatal (down) movements of test tooth by Muhlemann's micrometer gauge (50 gm. measured contact pressure against tooth).

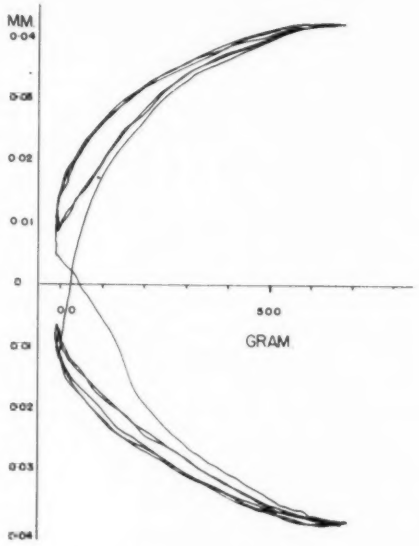

Fig. 4. Labial (up) and palatal (down) movements of test tooth measured by transducers (4 gm. contact pressure against tooth). Note return points 0.01 mm. short of zero due to friction against adjacent teeth.

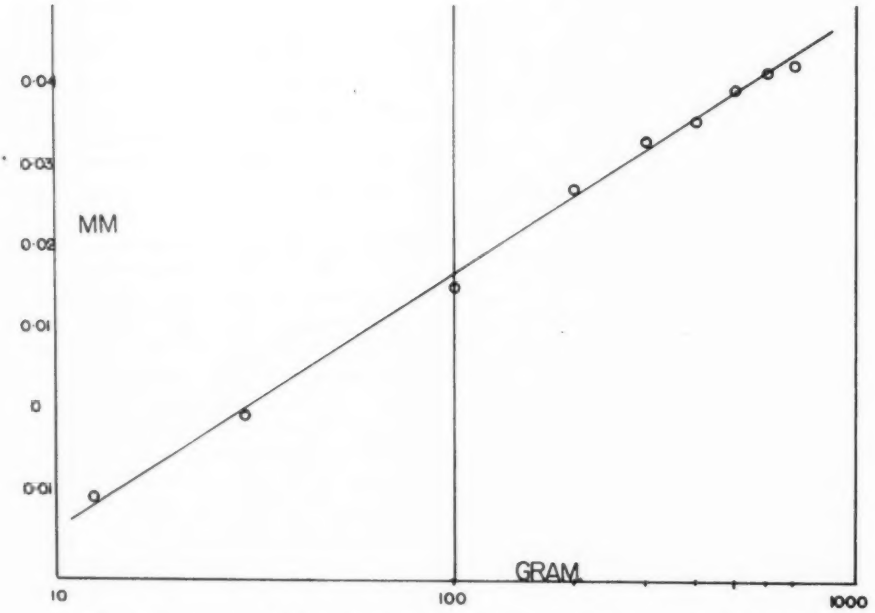

Fig. 5. Movement of initial labial movement (Fig. 4) plotted against log Force.

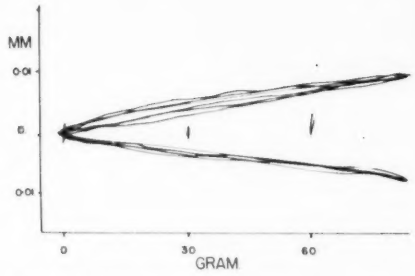

Fig. 6. Labial (up) and palatal (down) movement of test tooth measured by transducers in lower force range. Friction against adjacent teeth eliminated by discing.

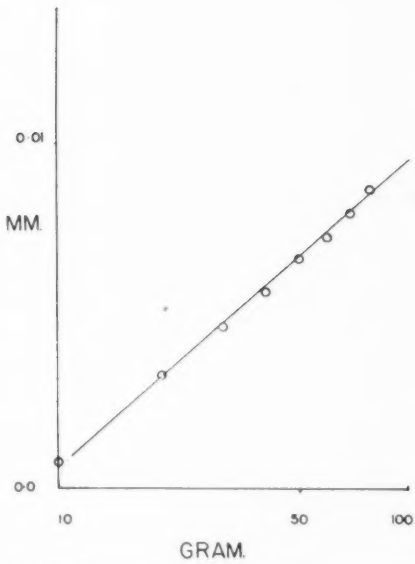

Fig. 7. Movement of initial labial movement (Fig. 6) plotted against log Force.

Fig. 8. Continuous time-movement record of labial movement of test tooth. (Record is read from below up as paper moves down.) F—Chewing one inch cube of steak in molar region on right and left sides. E—Biting stick of celery. D Chewing celery in premolar region. C—Biting apple. B—Chewing apple. A—Biting and chewing bread.

FINDINGS

A continuous recording of repeated force and movement made in the lingual and then the labial direction is shown in Figure 4. A maximum of 675 gm. was used. With this force this particular tooth moved 0.043 mm. labially and 0.037 mm. lingually. The movement in each direction closely followed a logarithmic function of the applied force (Fig. 5). This relationship was true in the lower range also (Fig. 6 and 7). Figure 7 shows that 0.007 mm. movement took place with 60 gm. of force.

Figure 8 shows the continuous time-movement record during the eating of various articles of food. Tough meat was chewed in the molar region; celery and apple incised and then chewed in the premolar region; and bread chewed in the incisor region. The least amount of labial movement of the incisor teeth took place when meat was chewed in the molar region, and the maximum amount of movement when celery was incised. During this procedure 0.052 mm. of labial movement of the upper incisor tooth took place. This latter indicates a labial force of more than 1 kg. Figure 9 shows a time-movement study with the teeth clenched in the most retruded position without food interven-

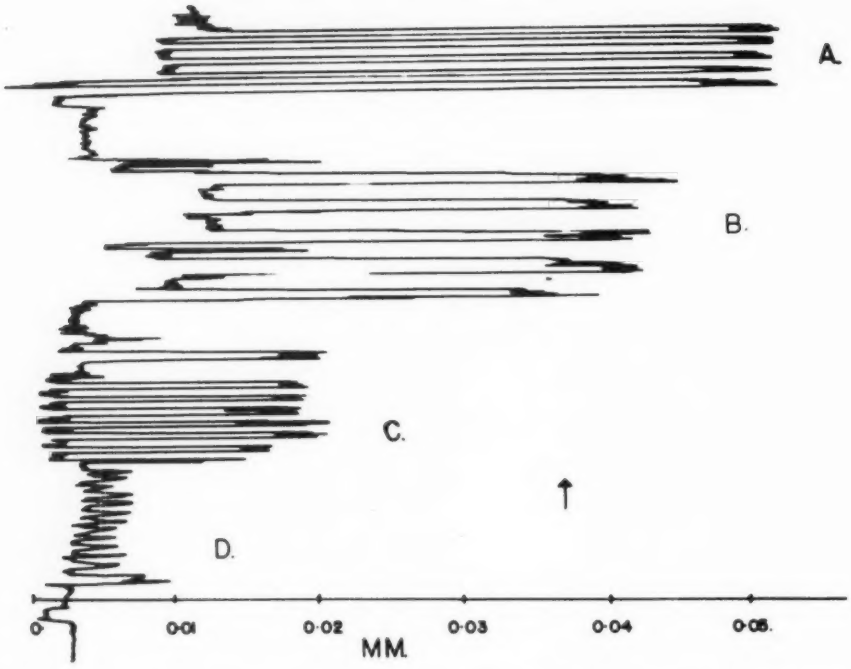

Fig. 9. Continuous time-movement record of labial movement of test tooth, clenching teeth without food intervening. D—In retruded position. C—Slightly forward of most retruded position, the habitual occlusal position of individual. B—Slightly protruded position, heavy occlusion on incisor teeth. A—Edge to edge bite on incisors.

ing, ½ mm. in front of the most retruded position (the normal occlusal position of this individual), in slight protrusion with the lower incisors on the lingual slopes of the upper incisors, and in the edge-to-edge position. The recorded labial movements of between 0.003 and 0.04 mm. indicate forces of between 10 gm. and 500 gm. in the labial direction.

Figure 10 shows a recording of the tongue being deliberately pushed against the palatal surface of the upper incisors, causing a movement of 0.02 mm., and also during swallowing movements. Figure 11 shows a time-movement record during speech. The tongue was placed against the upper incisors to make the following sounds: d, l, n, t, th. Labial movements of the upper incisor of 0.0025 mm. took place on making the sound "d", and 0.01 mm. with the sound "th".

DISCUSSION

Tooth mobility varies considerably with the individual. Mühlemann gives the normal range of 0.01 mm.-0.012 mm. for the upper incisor teeth. Pathologically loose teeth may move ten times, or more, this amount. There is a considerable individual variation in the occlusion of the teeth, strength of the tongue, and habits of phonation, mastication, and swallowing. The present measurements demonstrate the range of forces encountered and the degree of movement of a particular incisor tooth which was firmer than the average given by Mühlemann.

Throughout the body, functional movements assist the flow of blood and tissue fluid. Weight-bearing joints have avascular areas at the points of pressure, but the

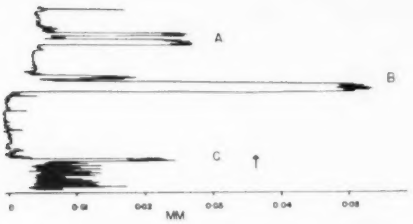

Fig. 10. Continuous time-movement record of labial movement of test tooth. C—Swallow after mastication of food followed by brief period of rest. B—Deliberate edge to edge bite. A—Deliberate tongue thrust against palatal surfaces of upper incisors.

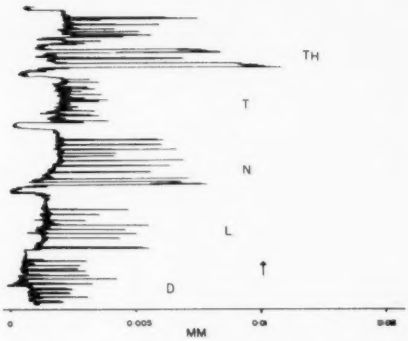

Fig. 11. Continuous time-movement record of labial movement of test tooth while repeating the sounds D, L, N, T, Th.

dento-alveolar joint, although bearing considerable weight per unit area comparable to other weight bearing joints, has an uninterrupted vascular plexus in the intervening tissue. Weight bearing in the dento-alveolar joint, however, is intermittent.

Axial movements of the tooth cause partial emptying of the vascual bed of the periodontal membrane.[5] No doubt lateral tipping of the tooth also causes localized areas of compression of the tissues. It may be speculated, therefore, that movements of the tooth within the socket may assist the circulatiton of blood and tissue fluid within the periodontal membrane, save the tooth from traumatic impact during occlusion, and increase bone absorption and replacement allowing the tooth to move away from trauma without severe increase in looseness. But when a tooth is excessively loose, in addition to damage caused by occlusal forces, movement is caused by the physiological forces of tongue and lips, which may bring about additional damage to the periodontal structures. When the tooth is excessively loose, violent movement may spread toxic substances from the crevice through the tissues. This may explain the clinical finding that pockets are often deeper and inflammation more severe beside loose teeth.

CONCLUSIONS

Since upper incisor teeth are subjected to axial forces which may rise to 15 kilograms during the incision of food, axial movement of the teeth of 0.04 to 0.05 mm. may take place, but the number of times during the day that this occurs is small compared with the number of labio-lingual movements, for the upper incisors are subjected to lingual forces not only during mastication of food, but with tooth clenching, swallowing, and speaking. These take place many thousands of times a day.

REFERENCES

1. Mühlemann, H.: Ten Years of Tooth Mobility. J. Periodont. 31:110-122, 1960.

2. Howell, A., and Manly, R.: An Electronic Strain Gauge for Measuring Oral Forces. J. Dent. Res. 27:705-712, 1948.

3. Kydd, W. L.: Maximum Forces Exerted on the Dentition by Perioral and Lingual Musculature. J.A.D.A. 55:646-651, 1957.

4. Anderson, D. J.: Measurement of Stress in Mastication. J. Dent. Res. 35:664-673, 1956.

5. Parfitt, G. J.: Measurement of the Physiological Mobility of Individual Teeth in an Axial Direction. J. Dent. Res. 39:608-618, 1960.

Healing of Human Periodontal Tissues Following Surgical Detachment From Vital Teeth: The Position of the Epithelial Attachment

by MELVIN L. MORRIS, B.S., M.A., D.D.S., *New York, New York*

STUDIES[11,12] of vital human teeth have shown that periodontal tissues reunite with both dentin and cementum after surgical separation. Marked new cemental deposits were frequently noted on dentin in root nicks at the apical end of the wounds. Although dentin was surgically exposed near the epithelial attachment a critical review of the microscopic sections did not show clear cut definitive removal of cementum up to and including this level. Since remaining bits of cementum on the root might easily influence the local healing reactions the experiments were repeated, this time carefully removing cementum up to this point.

PROCEDURE

The periodontal wounds and subsequent biopsies were performed as previously described.[11,13] Round burs, rather than curettes, were used to be certain of cemental removal. Post-operative periods ranged from 21 to 72 days.

Twenty-four cases are included in this study. Nine teeth were sectioned vertically and fifteen were serially sectioned in the horizontal plane.

RESULTS

I. *Vertical Sections:* Eight of the nine vertically sectioned teeth show rough shallow root concavities (Fig. 1) exposing the underlying dentin. The epithelial lining has grown part of the way, the apical end of the epithelial attachment resting on the dentin in a relatively shallow section of the excavation. Connective tissue fibers parallel to and in close apposition to the root are seen apical to the epithelial attachment. The epithelial lining has adapted itself to the surface of the deeper deformities by increasing its thickness.

One tooth (Fig. 2) showed a deeper more abrupt root concavity. In this case the epithelial lining grew past the denuded dentin and anchored on cementum. The epithelial cells lining the deeper parts showed marked vacuolization, a finding repeatedly observed in such areas when accompanied by marked cellular infiltrations. These deeper linings tend to thicken (as in Fig. 1) when the inflammatory reactions have been resolved.

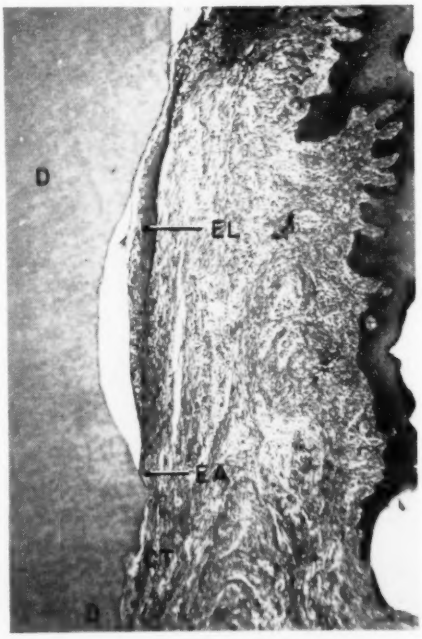

Fig. 1. Vertical section of a surgical pocket showing the epithelial lining (EL) stopping in an epithelial attachment (EA) on a shallow part of the surface of excavated dentin (D). Parallel connective tissue fibers (CT) are present apical to the epithelial attachment.

Associate Clinical Professor of Dentistry.

Section of Periodontology, Columbia University.

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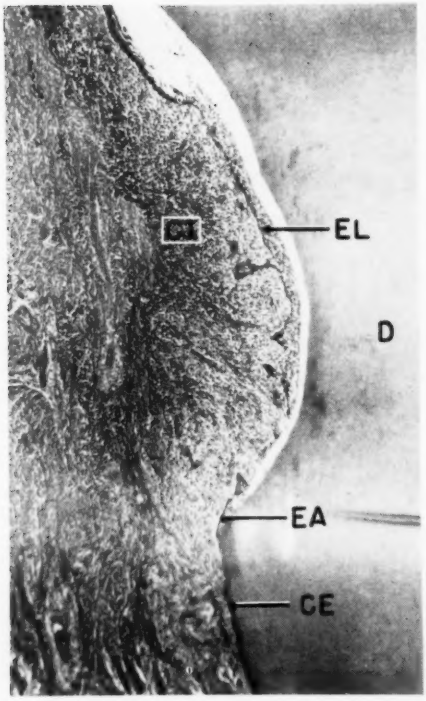

Fig. 2. Vertical section of a surgical pocket with a deep excavation of dentin (D). The epithelial lining (EL) passes denuded dentin stopping in an attachment (EA) on cementum (CE). Vacuolization of epithelial cells is noted. A marked cellular infiltration (CI) is present.

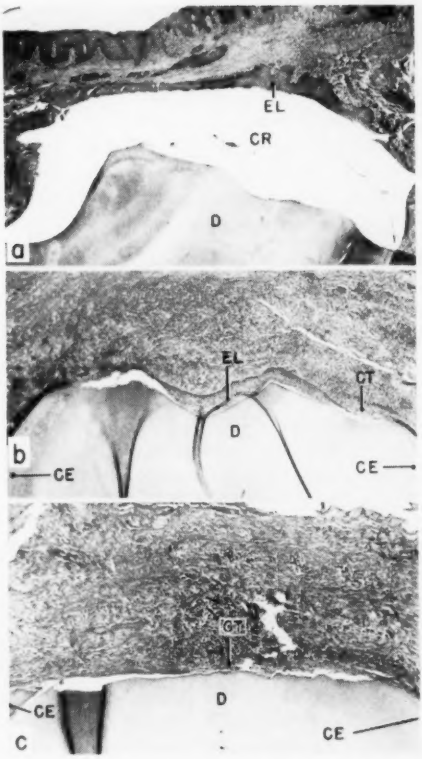

Fig. 3A. Horizontal cross section of gingival crevice (CR) with epithelial lining (EL) opposite exposed dentin (D).

Fig. 3B. Section apical to 3A. The epithelial lining (EL) remains where more dentin (D) was removed, the lining being thicker where deeper. Connective tissue (CT) union is present at the lateral borders where only small amounts of dentin and cementum (CE) have been removed.

Fig. 3C. Section apical to 3B. The dentinal grooves have flattened out with a shallow surface of dentin (D) bordered by connective tissue (CT) with fibers parallel to the tooth.

II. *Horizontal Serial Sections:* Epithelium ended in all sections where the root deformity became relatively shallow and broad, without regard for the histologic nature of that terminal point.

Moving apically through the serial sections the epithelial lining of a crevice (Fig. 3A) may be seen to narrow as it reaches the area of surgical detachment (Fig. 3B) where cementum was partly removed at the lateral borders and completely removed with some dentin toward the center. This central dentinal surface is covered with an epithelial lining, typically thicker in the deeper spots, while the shallower side portions have a connective tissue covering.

Further down, the dentinal grooves merge to become a single one, broad and shallow (Fig. 3C). Here connective tissue union occurs, stopping epithelial progress, the fibers being parallel to the root surface.

The next example (Fig. 4A) shows a crevicular epithelial lining which stops (Fig. 4B) as the untouched cementum in the area of surgical detachment is reached but continues its downward plunge in a cemental groove until it flattens out (Fig. 4C). Again, the fibers are parallel to the root surface.

In Figure 5A two deep parallel grooves are noted with an epithelial lining covering the grooves and the cementum in between, and invading a tiny undercut dentinal groove. Lower down (Fig. 5C) all cementum has been removed, the shallow center section showing a connective tissue union while flanking columns of epithelium persist in the two deep lateral dentinal grooves. The connective tissue fibers have assumed a direction parallel to the root surface.

DISCUSSION

Every repair process involves, to some degree, a re-enactment of the embryological process of cellular differentiation. It seems reasonable to assume that chemical inducing substances influence the differentiation in repair as they are known to do in embryological development. Applying this concept to the present experiments the root would present the inducing surface and the periodontal tissues would contain the differentiating cells.

Among the conditions necessary for the success of this process are the presence of inductive substances, cells that are capable or competent of responding to these stimuli, and an intimate contact between inducing and responding systems.[2]

The results in this experiment seem to highlight the importance of the last condition, namely, intimate contact. In every case connective tissue union began (and epithelium ended) where the artificial root deformity became very shallow or terminated abruptly. At these points it was possible for periodontal tissues to come into close contact with the root. This, theoretically, brought the competent responding cells under the inductive influence of the root.

It did not matter whether the root at that point was covered with dentin or cementum. This was amply illustrated by epithelial linings that stopped on dentin (Fig. 1, 3, and 5), passed exposed dentin to stop at the first cementum (Fig. 2), or continued apically for a distance on cementum (Fig. 4).

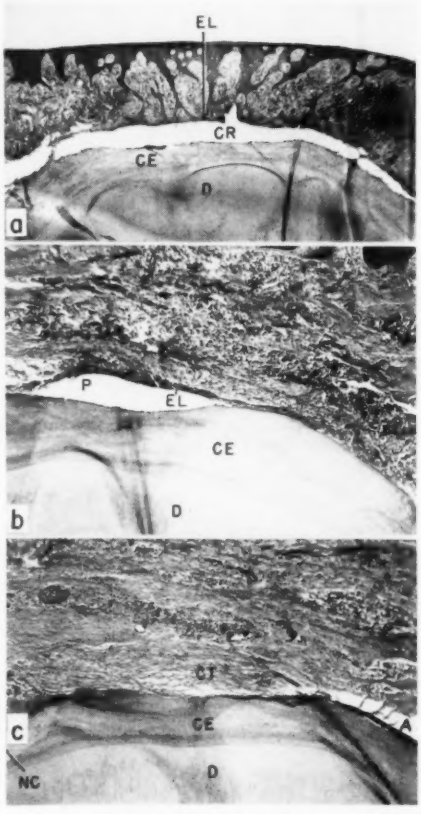

Fig. 4A. Horizontal cross section of gingival crevice (CR) bordered by an epithelial lining (EL) and cementum (CE).

Fig. 4B. Section apical to 4A. Connective tissue (CT) borders cementum (CE) on lateral border but epithelial lining (EL) persists and forms pocket (P) where cementum has been grooved.

Fig. 4C. Section apical to 4B. The cemental groove has flattened out and now connective tissue (CT) has formed on cementum (CE) to stop further epithelial downgrowth. The collagenous fibers are parallel to the root surface.

New cementum (NC) is seen in left corner, forming in a cemental groove which had been made apical to the level at which healing had occurred, and was thus protected against an interfering epithelial downgrowth.

A is an artefact.

Our previous studies had seemed to indicate that epithelial growth was halted on vital teeth[11,12] when the first surgically exposed dentin or cementum was met, but

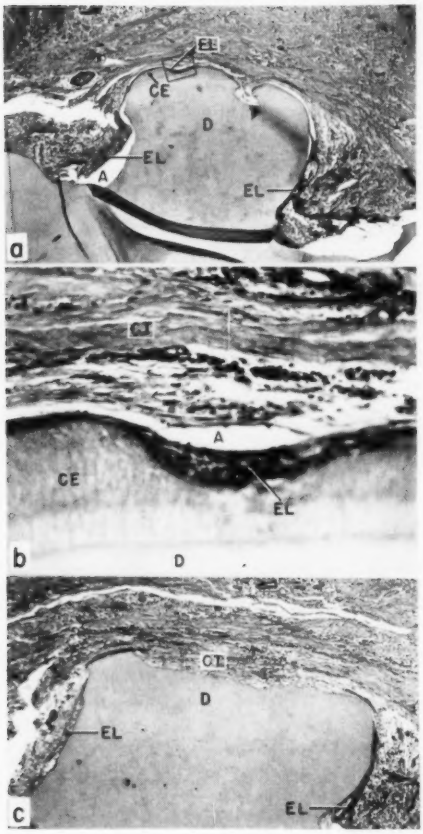

Fig. 5A. Horizontal cross section of surgical pocket with two deep lateral grooves in dentin (D). Epithelial lining (EL) persists on both dentin and cementum (CE).

Fig. 5B. Higher magnification of inset in 5A, showing the epithelial lining (EL) on cementum (CE). (CT) is connective tissue, (A) is artefact, and (D) is dentin.

Fig. 5C. Section apical to 5A and 5B. The root surface of the wound is now only dentin (D). Connective tissue fibers (CT) are parallel to and in contact with the shallower dentin while the two deep flanking grooves are still lined with epithelium (EL).

continued on past exposed dentin only in non-vital teeth[13,15] until the first cementum was reached. A reappraisal shows that these apparent differences can now be largely explained on the same morphological basis as in the present study. It is evident that interpretation of results in non-vital teeth must take into account anatomic as well as chemical differences.

It also explains why epithelium grew past denuded dentin in Stones'[18] experiments on vital monkey teeth where the deep deformities looked exactly like Figure 2.

That an epithelial attachment may be halted on dentin without a layer of cementum to prevent further invagination has also been reported, in animals, by Jansen and co-workers[10] in wound studies and in Waerhaug's investigation[19] of experimental traumatic occlusion.

Union did not take place where there was a wide space between the gingival tissue and root. Yet healing does occur across similar spaces when they are remote from the gingival crevice[3,4,5,6,7,8,10,11,12,13,15,16,17] and its accompanying epithelium. If epithelium had simply exerted an inhibitory effect it would have also prevented healing where the root deformity was shallow. The results can be more logically explained by picturing a process too slow to be completed before epithelial downgrowth. Wider spaces contain thicker clots requiring more time to be replaced by competent connective tissue elements. The blood clot has long been considered an integral part of the "reattachment" process. It is interesting to speculate on the possibility that, under the special conditions of a periodontal wound, where one side is vascular and the other avascular, a clot may be a hindrance that must be eliminated before union is consummated. This is similar to Ham's[9] concept of the clot in bone fracture healing.

It now becomes possible that the continued integrity of the supra-alveolar periodontal attachment may be somewhat dependent on how well the muscles of tongue, facial expression and mastication keep the soft tissue in close adaptation to the root. This would insure prompt healing if local irritations caused a temporary breakdown in the periodontal fibers. If tiny narrow vertical root grooves (similar to that in Figure 4) existed they would tend, after separation, to allow narrow deep tongues of epithelium to grow down. These

would result in pockets which could account for the occasional narrow vertical extensions of calculus noted by Everett and Potter.[1] If this hypothesis were correct, firm muscular pressure would help to prevent apical migration of the epithelial attachment as well as discouraging coronal growth of the free gingivae.[14] The net effect would be minimal crevice depth.

Further studies are in progress on the importance of firm tissue adaptation in periodontal healing.

It was also noted that connective tissue fibers appeared parallel to the root surface in both vertical and horizontal sections. There evidently is a mingling of fiber groups at angles to each other, all being parallel to the root surface. This observation is being amplified and developed in a forthcoming paper.

SUMMARY

1. In periodontal wounds in human vital teeth the epithelial attachment is halted by a connective tissue union at the first point of close contact between periodontal and root tissues.

2. It does not matter whether the root surface at the point of contact is cementum or dentin.

3. It is possible that a blood clot may delay this process long enough to permit epithelial invagination.

Appreciation is expressed to the many who have helped to complete this study. This includes Dr. Frank E. Beube, Dr. William Savoy, Dr. J. Anderson, Dr. H. Baurmash, Dr. G. Minervini, Dr. P. Vahdat and Mrs. F. Moore, Mrs. R. Feinman, Miss E. Boyd, and Mrs. B. Coffiner.

BIBLIOGRAPHY

1. Everett, F. G., and Potter, G. R., "Morphology of Submarginal Calculus," J. Perio., 30, 27-30, 1959.

2. Grobstein, C., "Inductive Tissue Interaction in Development," Advances in Cancer Research, 4, 187-236, 1956.

3. Beckwith, T. D. et al: "The Regeneration of Rodent Periodontal Membrane," Proc. Soc. Exp. Biol. and Med. 24:562-64, 1927.

4. Beckwith, T. D., and Williams, A.: "Regeneration of Periodontal Membrane in the Cat," Proc. Soc. Exp. Biol. and Med. 25:713-15, 1928.

5. Beube, F. E., and Silvers, H. F.: "Influence of Devitalized Heterogeneous Bone Powder on Regeneration of Alveolar and Maxillary Bone in Dogs," J.D. Res. 14:15-19, 1934.

6. Beube, F. E., and Silvers, H. F.: "Further Studies on Bone Generation with the Use of Boiled Heterogeneous Bone," J. Perio., 1:17-21, 1936.

7. Beube, F. E., "A Study of Reattachment of the Supporting Structures of the Teeth," J. Perio., 18:55-66, 1947.

8. Beube, F. E., "Factors in the Repair of Alveolar Bone and Cementum," Oral Surg., Oral Med., Oral Path., 2:379-403, 1949.

9. Ham, A. W., in Bourne's, "The Biochemistry and Physiology of Bone," Academic Press Inc., New York, 1956.

10. Jansen, M. T. et al., "The Healing of Periodontal Wounds in Dogs," J. Perio., 26, 292-300, 1955.

11. Morris, M. L.: "The Reattachment of Human Periodontal Tissues Following Surgical Detachment: A Clinical and Histological Study," J. Perio., 24, 220-228, 1953.

12. Morris, M. L.: "Healing of Naturally Occurring Periodontal Pockets About Vital Human Teeth," J. Perio., 26, 285-292, 1955.

13. Morris, M. L., "Healing of Human Periodontal Tissues Following Surgical Detachment from Non-Vital Teeth," J. Perio., 28, 222-238, 1957.

14. Morris, M. L., "The Position of Margin of the Gingiva," Oral Surg., Oral Med., Oral Path., 11, 969-984, 1958.

15. Morris, M. L., "Healing of Human Periodontal Tissues Following Surgical Detachment: Extirpation of Vital Pulps," J. Perio. (In Press).

16. Noyes, F. B.: "A Report on Experiments to the Periodontal Membrane of Dogs," Dent. Rev., 28:883-91, 1914.

17. Sippy, B. O.: "Regeneration of Tissues Following Injury of Tooth Roots," D. Cos., 69:771-80, 1927.

18. Stones, H. H., "The Reaction and Regeneration of Cementum in Various Pathological Conditions," Proc. Roy. Soc. Med., 27, 728-744, 1934.

19. Waerhaug, J., "Pathogenesis of Pocket Formation in Traumatic Occlusion," J. Perio., 26, 107-118, 1955.

The Microhardness of Cementum and Underlying Dentin of Normal Teeth and Teeth Exposed to Periodontal Disease

by C. A. Rautiola* and R. G. Craig, *Ann Arbor, Michigan*

Curettage and root planing have been basic procedures in the treatment of periodontal pockets, although the physical properties of the cementum and underlying dentin of teeth involved in periodontal disease only recently have been studied.[1,2] Clinicians, however, have maintained that cementum which is exposed to a periodontal pocket is infected or soft, and should be removed during curettage and operations performed in attempts to attain reattachment.[3-13] The thickness of cementum which should be planed away is in some dispute. However, there is apparent agreement among periodontists that the portion which is soft to clinical perception should be removed.

Riffle[9,10,14,15] found that cervical areas of teeth, the cementum, and the underlying dentin, were soft when extracted teeth from patients experiencing periodontal disease were curetted. A Kingsley vulcanite scraper was used on the extracted teeth until they felt smooth. This procedure resulted in gross deformities in the cervical areas of teeth. Thus it was concluded that diseased or exposed cementum and dentin were soft. The degree and depth of softness were found to vary directly with the age of the patient and the depth of the pocket.

The existence of radiolucent zones in the cervical areas of teeth has been advanced as evidence for the contention that these cervical areas are hypocalcified and soft. However, Raper[16] explained these zones on the basis of variations in the angulation of the x-ray beam rather than on the presence of pathology. Maden and Brudevold[17] have demonstrated that the radiolucent zones at the cervical areas of teeth may be explained on differences in morphology, angulation of the x-ray beam, and the height of the alveolar crest.

Röchert[18] studied the amount of calcium in different areas of the cementum of rhesus monkeys, using a spectroscopic technic and found no significant differences among teeth or in different areas of a single tooth. The periodontal condition of the teeth, however, was not reported.

Stones[19] reported that acellular cementum, in which Sharpey's fibers are calcified was impermeable to dyes. Bartelstone[20] and Wainwright[21] observed that radioactive iodine could permeate cementum and dentin, and they concluded that cementum exposed to a periodontal pocket and the underlying dentin were more permeable than cementum from areas adjacent to a normal periodontal ligament.

Kronfeld[22] and Thoma and Goldman[23] have suggested that the inflammation associated with periodontal pockets is the cause of cemental changes such as resorption. More recently, Henry and Weinmann[24] examined sections of material obtained at autopsy and found that there were no resorptive changes in cementum which were associated with inflammation adjacent to periodontal pockets, granulomas, or cysts.

Harvey and Zander[25] studied extracted teeth and observed that teeth with periodontal disease showed three times more resorption of root surface than normal teeth. The areas of increased resorption occurred most often in the apical one-third of the

A portion of this work was supported by a National Science Foundation Summer Teaching Fellowship and is from the thesis of Clifton A. Rautiola, submitted in partial fulfillment of the requirements for the Degree of Master of Science in Periodontics at the University of Michigan.

*Present Address: 1110 Bank of Lansing Building, Lansing, Michigan.

roots of the teeth. This indicated that cemental resorption was not entirely due to inflammation.

Zander and Hürzeler[26] studied the thickness of cementum and showed that thickness varied directly with the age of the tooth in a straight line relationship, having an average thickness of 0.1 mm. at age 20 and increasing to 0.2 mm. at age 55.

There is a paucity of information on hardness values for cementum. Hodge McKay[27] used a scratch test and reported that cementum had a mean range of 85 to 90 Bierbaum hardness numbers. More recently, Ichiro[2] using a Vickers hardness tester, observed mean hardness values for adult human cementum to range from 14 to 21 diamond pyramid hardness numbers. No data were presented regarding the anatomical location or periodontal condition of the teeth studied.

The microhardness of dentin has been studied by several different investigators.[1,27-30] However, microhardness values for peripheral dentin, which lies immediately beneath the cementum, have not been reported. Emslie and Stack[1] investigated the microhardness of dentin in normal teeth and teeth with periodontal disease.

Martens, Bradford, and Frank,[31] after reviewing the literature, concluded that transparent dentin may be hypercalcified and/or hypocalcified. The transparent dentin may be produced by age and maturation changes which result in a calcific material replacing the originally soft content of the dentinal tubules. Nalbandin[32] found that electronmicrographs of decalcified sections of dentin from sclerosed and transparent roots suggested the presence of a highly acid soluble zone in the peritubular regions of the dentin. Microradiographs of extracted teeth were used to show that a high degree of tubular obliteration, due to dense mineral deposition, occurred in transparent dentin.

The preceding brief review of studies involving the properties of cementum and dentin suggested that an investigation of the hardness of cementum and underlying dentin of normal teeth and teeth with periodontal disease would yield information concerning the condition of these tissues. Thus, it was felt that it might be possible to relate microhardness values to the previously reported physical properties of cementum and to the clinical procedures of root planing and scaling.

METHODS AND MATERIALS

Specimen Preparation. Freshly extracted teeth were obtained from patients along with biographical data and the depth of the periodontal pocket. Patients were selected on the basis of no previous history to scaling. Eighteen teeth were used in this study, four of which were embedded directly in casting plastic within 20 minutes after extraction. The remaining fourteen teeth were immersed and stored in neutral formalin until embedded in plastic. The teeth were cast in plastic, sectioned, ground, and stored in water as previously reported by Craig and Peyton.[29] Storage in tap water, neutral formalin, or direct embedding into plastic had no apparent effect on microhardness of dentin and cementum.

Either longitudinal or surface sections were prepared, as indicated in Figures 1 and 2, respectively. Extended polishing of sections containing areas of different hardness, such as cementum and dentin, produced sloping surfaces at the junction. This problem was minimized by polishing the sections for a maximum of 20 seconds on a polishing cloth using C-RO metallographic polishing compound.

Microhardness Testing. An "MO" Tukon hardness tester was used with a Knoop diamond indenter. A 25 gram load and a 15 second contact time were used throughout the study. For purposes of standardization, several hardness values were determined each day for central crown or root dentin. Any variation in hardness from 65 $\pm$ 10 KHN prompted

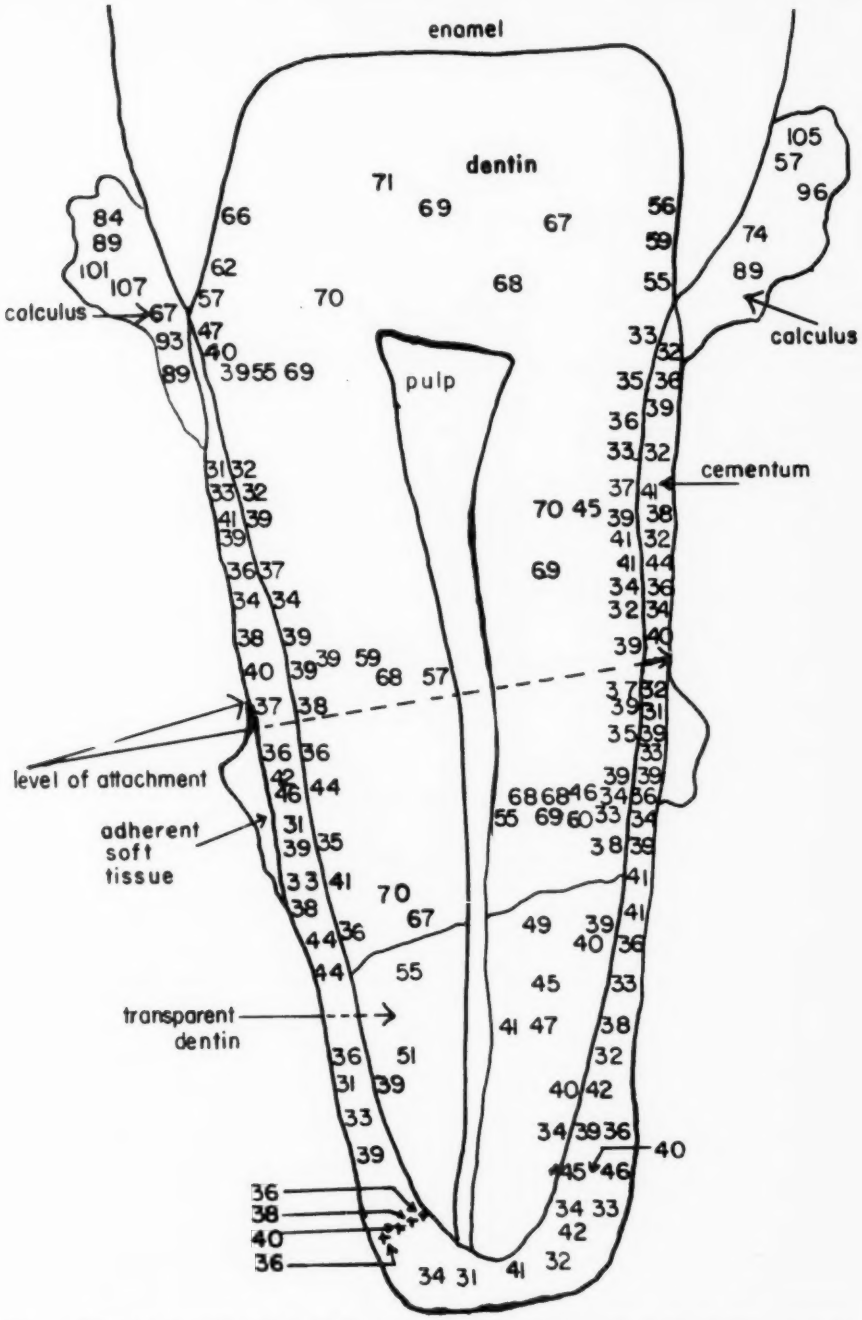

Fig. 1. Schematic drawing of a longitudinal section showing representative Knoop hardness numbers for different areas of a tooth.

a check of the equipment to eliminate mechanically induced errors. Indentations were accepted on the basis of the proper long to short diagonal ratio and/or the observed symmetry. A total of 1640 indentations were made into various locations of 26 sections of the 18 different teeth.

Surface sections and longitudinal sections were prepared for several teeth. The microhardness of cementum was determined on the surface sections as well as the longitudinal sections. No difference was observed in the hardness of cementum from surface sections and longitudinal sections. Therefore, since longitudinal sections were more satisfactory for testing purposes, all subsequent tests were performed on longitudinal sections.

Microscopic Measurements. The epithelial attachment was discernible microscopically on some sections and the adjacent connective tissue attachment was easily distinguished in all sections. When the epithelial attachment was present on the root surface, it was used to delineate the exposed cementum from the unexposed cementum. Otherwise, the connective tissue attachment was used as a reference point. It may be argued that a discrepancy exists between epithelial and connective tissue attachment levels. Some pockets were very deep and one tooth had an 8 mm. pocket. This distance greatly exceeds any possible length of an epithelial attachment.

RESULTS

Cementum. A comparison of microhardness of surface cementum and sectioned cementum is shown in Table I. Tooth A^1, which was sectioned longitudinally, had similar hardness readings for comparable areas to that of tooth A^2. Figures 1 and 2 also show that no difference was observed between the microhardness of surface and sectioned cementum.

The microhardness values for cementum of all teeth studied are listed in Table II.

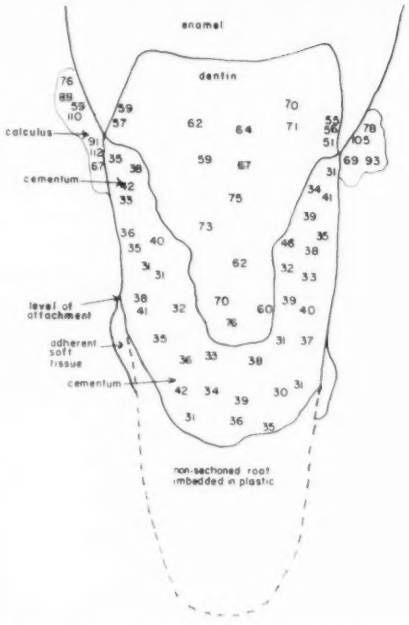

Fig. 2. Schematic drawing of a surface section showing representative Knoop hardness numbers in the surface of cementum.

The data indicate that exposed and unexposed cementum are of equal hardness. For all teeth the mean KHN was 39.6 (σ6.1) for unexposed cementum and 39.3 (σ5.3) for exposed cementum. Closer analysis of Table II shows that even though there is a variation as great as 7 KHN between exposed and unexposed cementum on tooth 0^1 the level of significance is approximately 30%, and therefore, in this most favorable case there is essentially no indication of a difference between the hardness of exposed and unexposed cementum. The indentations shown in Figure 3 also illustrate that no measurable difference was observed in the microhardness of exposed and unexposed cementum.

The values in Table II also suggest that there was no trend in hardness with respect to the age of the patient or to the depth of the periodontal pocket.

Extremely wide variations in hardness

TABLE I
Comparison of KHN for Surface Cementum and Longitudinally Sectioned Cementum

Preparation of Specimen	*Unexposed Cementum*			*Exposed Cementum*		
	KHN	*No. Read.*	*Std. Dev.*	*KHN*	*No. Read.*	*Std. Dev.*
Sectioned (A^1)	40	13	5.6	36	10	5.9
Surface (A^2)	37	25	6.2	36	9	5.1

were observed among different locations of the same tissue of the same tooth. Some teeth, however, showed little variation in hardness among different locations. These variations account for the large standard deviations reported for some teeth and low standard deviations for other teeth. The nature or cause for the variation in hardness was not evident from the results of this study.

In some teeth the cementum was sufficiently thick so that several indentations could be placed between the surface of the cementum and the cementum-dentin junction. Microhardness values for outer and inner cementum of 8 teeth are listed in Table II. The mean KHN for outer cementum was 41 ($\sigma 6$) and for inner cementum was 39 ($\sigma 7$). The difference between the means is not statistically significant, and therefore, outer and inner cementum are not different in hardness apart from the normal wide variations in hardness found throughout cementum. This conclusion is illustrated by Figure 4 which shows Knoop indentations in outer and inner cementum.

There was a marked variation in the thickness of cementum among the different areas of the root. There appeared to be a general increase in thickness near the apex of all the teeth studied. Except for the extremely thin cementum (in some instances less than 4 microns) near the cemento-enamel junction, the cementum covering most of the root was fairly uniform in thickness, varying from 15 to 30 microns. Near the apex, the cementum became markedly thicker and increased to approximately 150 microns.

A difference in elastic recovery was observed between cementum and dentin. Indentations placed in cementum were nearly obliterated by elastic recovery within several hours, while indentations placed in dentin remained nearly intact for at least six months.

Dentin. The microhardness of peripheral dentin under cementum and enamel, central root and crown dentin, and apical transparent dentin are reported in Table III. A limited number of hardness values are also reported for calculus.

The dentin, excluding peripheral root and crown dentin, was found to have a mean KHN of 69 ($\sigma 7$). This dentin is called central root and crown dentin to distinguish it from peripheral dentin which had different hardness values.

The mean KHN for a narrow band of peripheral dentin between the central root dentin and the cementum was 38. This narrow band of soft peripheral dentin varied from 60 to 130 microns in thickness and in general measured approximately 100 microns. The soft peripheral dentin was found in all teeth and as illustrated in Figure 1 had a comparable hardness to the adjacent cementum.

Peripheral dentin immediately underneath the enamel generally was softer than central coronal dentin. The mean KHN for peripheral crown dentin was 61. The peripheral dentin covered by enamel was much harder than that covered by cementum. This change in hardness of dentin under cementum and of dentin under

TABLE II
Microhardness Values for Cementum

Tooth	*Distance from C-E Junct. to Attach., mm.*	*Age of Patient*	*KHN of Unexp. Cementum*	*Standard Deviation*	*No. of Readings*	*KHN of Exp. Cementum*	*Standard Deviation*	*No. of Readings*	*KHN of Outer Cementum*	*KHN of Inner Cementum*
A[1]	2.5	55	40	4.6	16	36	5.1	29	42	37
A[2]	2.5	55	37	5.6	19	36	4.2	9		
B[1]	4.5	61	27	3.7	9	29	8.0	13		
B[2]	4.0	61	32	2.4	9	32	2.1	7		
C	6.0	61	32		27	28	2.4	10	32	34
D	0.0	30	40		26					
E	0.0	25	50		5					
F	0.0	50	34		13					
G	0.0	31	32		20					
H*	6.5	61	36	3.8	14	38	3.8	18		
I[1]*	3.5	36	44	3.3	18	39	3.6	16	41	42
I[2]*	3.0	36	38	4.6	25	42	5.6	14		
J[1]*	3.5	52	43	2.8	19	40	6.1	13		
J[2]*	3.5	52	35	5.6	20	43	2.1	9		
K[1]*	5.0	52	42	3.0	8	37	4.4	38	42	43
K[2]*	5.5	52	33	3.2	4	33	1.2	34		
L[1]	8.0	22	43	2.0	7	44	3.5	20		
L[2]	4.5	22	42	2.1	6	47	3.9	16		
M	1.0	40	43	6.8	45	47	1.2	6	35	46
N[1]	3.5	40	42	8.5	20	45	5.9	29		
N[2]	1.0	40	37	7.8	49	40	2.6	6	31	39
O[1]	5.0	46	49	6.0	4	42	2.4	16		
O[2]	4.5	46	49	6.0	7	44	1.7	10	52	46
P	7.0	46	50	3.0	4	48	5.1	14		
Q	0.5	33	44	8.0	52	38	7.0	5		
R	2.5	55	38	5.3	57	37	4.1	16	40	41
Mean			39.6	6.1		39.3	5.3		41.0	39.4
Std. Dev.									5.6	6.8

*Specimens were embedded directly in plastic; remaining teeth stored in formalin.

enamel is shown in Fig. 1, where the peripheral dentin changes from 32 to 34 under cementum to 55 to 66 under enamel. Indentations in the peripheral dentin near the cemento-enamel junction are shown in Figure 5, as well as indentations in central crown and root dentin. The sizes of the indentations clearly show that the peripheral dentin under cementum is softer than dentin underlying enamel, and central crown and root dentin.

A limited number of indentations were placed in the peripheral dentin, adjacent to the pulp. The hardness values which were obtained indicated a soft zone of dentin near the pulp. The Knoop hardness of peripheral pulpal dentin was 10 to 15 KHN less than central crown and root dentin.

Fig. 3. Knoop indentations in cementum, C, and dentin, D. The epithelial attachment is at A. The indentations show no difference in hardness between exposed and unexposed cementum (150X).

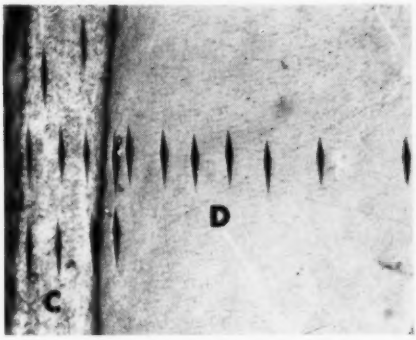

Fig. 4. Knoop indentations in cementum, C, and dentin, D. The indentations show no difference in hardness of inner and outer cementum, and no difference between cementum and outer peripheral dentin. The dentin was transparent in this area and had a KHN of 48 (180X).

Transparent dentin in the apical one-third of the roots of some teeth were observed to be much softer than normal-appearing dentin of the same tooth. The transparent dentin had a range of KHN's from 40 to 47. These values are abnormally low when compared to the mean value of 69 KHN for central crown and root dentin.

Transparent dentin occurred in teeth from patients older than 50 years of age, except one. In this instance, the patient was 33 years of age, but the tooth was a lower incisor which had gingival recession halfway down the root, with a further detachment and pocket formation extending to within 1.5 mm. of the apex of the tooth. The tooth also was in traumatic occlusion.

Calculus. Calculus was attached to four teeth in sufficient quantity to permit hardness indentations. A mean KHN of 86 (σ4) for calculus is reported in Table III. Individual readings showed a wide variation from 64 to 110 KHN. Since areas of uncalcified matrix were present within the calculus, care was taken to select areas which were calcified and large enough to contain the entire indentation. Readings in the soft matrix areas were valueless since an adequate indentation for measurement could not be obtained.

DISCUSSION OF RESULTS

Comparison of the Knoop hardness values of cementum with other previously reported microhardness values is difficult since different testing devices and units of measurement were used. Hodge and McKay[27] found cementum to have a value of 85 to 90 Bierbaum hardness numbers but a comparison is not possible since these numbers were obtained by a scratch test. Ichiro[2] used a Vickers hardness tester and reported values for cementum from 14 to 21 diamond pyramid hardness numbers (DPH). Generally, Vickers and Knoop hardness numbers agree quite well and the lower values obtained by Ichiro are difficult to reconcile with the mean KHN of 39 which was obtained in the present study.

The mean KHN of 39.3 (σ6.1) for unexposed cementum compared to 39.6 (σ5.3) for exposed cementum indicated that the microhardness was unaltered by exposure to a periodontal pocket or by ex-

posure following gingival recession.

Inner cementum was not different in hardness from outer cementum based on mean values of 39 and 41 KHN's respectively. In some instances inner cementum was found to be softer than outer cementum and in other cases the reverse situation occurred. This may be explained by noting the normal variation in hardness found in all areas of cementum and by recalling that acellular and cellular cementum are not constant in location but alternate from inner to outer in a random arrangement.[33]

The findings of Riffle,[9,10,14,15] that cervical areas of teeth were grossly soft when exposed to periodontal disease, are difficult to reconcile with the microhardness values of the present study. Riffle observed hardness by resistance to abrasion while the current study used a standard microindentation hardness procedure. Although hardness results cannot be compared strictly with abrasion values, some degree of correlation usually exists.

The microhardness values for cementum tend to confirm the findings of Maden and Brudevold,[17] and Raper,[16] that radiolucent zones in the cervical areas of teeth were actually artifacts. The results of the present study also substantiate the observation of Röchert[18] that the calcium content of cementum did not vary from one area to another. The increased permeability of exposed cementum, reported by Bartelstone[20] and Wainwright,[21] is difficult to interpret in light of the present study.

It is apparent that even though clinicians have long maintained that cementum which is exposed to periodontal pockets is soft, the results of the present microhardness studies of cementum seem to dispute this contention.

The results of the present investigation and those of Craig, Gehring, and Peyton[30] show that the microhardness of dentin must be described in terms of the location in dentin. The hardness of central root

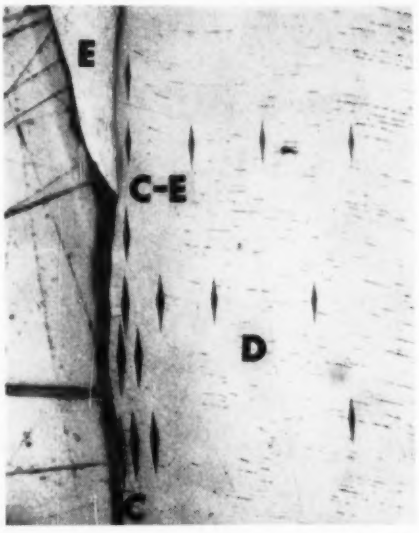

Fig. 5. Enamel, E, cemento-enamel junction, C-E, cementum, C, and dentin, D, are pictured. The indentations show the softer peripheral dentin covered by enamel and cementum, and the abrupt change in hardness of dentin at the cemento-enamel junction (150X).

and crown dentin of 69 (σ7) KHN is in good agreement with the value of 68 ± 3 KHN found by Craig and Peyton.[29] Both of these studies found peripheral dentin under enamel to be softer than central root and crown dentin, although the present investigation recorded a mean difference of 8 KHN compared to a difference of 15 KHN in the former study.

Peripheral dentin under cementum was much softer than central root and crown dentin. Actually, there is no difference in hardness between cementum and peripheral root dentin as indicated by the mean value of 39 KHN for cementum and 38 for peripheral root dentin. The presence of this softer zone of dentin, which was found to be approximately 100±30 microns in width, has not been reported previously. This zone may be associated with the granular layer of Tomes. Peripheral dentin at the cemento-enamel junction showed an abrupt change in hardness, being approximately 38 KHN under cementum and 61 KHN under enamel. The

TABLE III
Microhardness of Dentin and Calculus

Tooth	*KHN of Central Root and Crown Dentin*	*KHN of Peripheral Dentin under Cementum*	*Standard Deviation*	*No. of Readings*	*KHN of Peripheral Dentin under Enamel*	*KHN Transparent Dentin*	*KHN of Calculus*
A[1]	60	35	1.4	7	55	40	82
A[2]	61	33	1.3	10	57		78
B[1]	71	35	6.9	14			
B[2]	71	35	2.3	16			
C	69	29	5.0	7	60		86
D	70	43	2.6	4			
E							
F	69						
G	71	32	1.0	3			
H	68	39	1.1	6		43	
I[1]	80	42	3.1	5	71		91
I[2]	80						
J[1]	60	38	3.2	12	45	44	
J[2]	59						
K[1]	52	34	5.2	13	50	40	
K[2]	61						
L[1]	68	38	4.5	10	59		
L[2]	68						
M	75	43	3.9	4	76		
N[1]	69	55	7.8	11	71		
N[2]	69						
O[1]	71	46	3.1	6	64		
O[2]	71						
P	69	40	7.5	11	67		
Q	57	39	8.9	9	68	47	
R	64	33	4.2	12	55		79
Mean	68.8	38	6.1		61	43.0	86.0
Std. Dev.	7.3					3.0	4.2

reason for this dramatic change in hardness at the cemento-enamel junction was not apparent from the data of this study.

Transparent dentin in the apical one-third of a root with periodontal disease was much softer than normal-appearing dentin in the same tooth. The difference in microhardness was approximately 25 KHN. Transparent dentin has been described by Martens, Bradford, and Frank[31] as being either hypercalcified or hypocalcified. However, Nalbandin has reported transparent dentin to result from increased calcification as evidenced by a highly acid soluble peritubular region. As the tooth became more transparent, there was a simultaneous decrease in the number of tubules. It is difficult to reconcile the measured decrease in microhardness of transparent dentin in the present study

with the observed increase in calcification of Nalbandin's study.

The observed cementum thickness ranged from 5 to 150 microns. Most of the cementum was from 15 to 30 microns thick except for the extremely thin cervical area and the thicker apical area. These values tend to show cementum to be thinner than reported by Zander and Hürzeler.[26]

No difference in dentin hardness was observed resulting from periodontal disease. Emslie and Stack[1] reported a difference in hardness of peripheral dentin near the pulp which was caused by periodontal disease. However, the microhardness difference which they reported was very small considering the normal wide variations observed in dentin in this and other studies.[27,29,30] This wide variation in values was not reported by Emslie and Stack.

The limited measurements on the hardness of calculus indicate that this material is somewhat harder than dentin and much harder than cementum. The average value of 86 KHN for calculus when compared to 39 KHN for cementum may explain why root hypersensitivity exists following periodontal treatment. In attempting to remove the hard calculus, the soft cementum and peripheral dentin perhaps are removed. This would very likely be the case if root planing were carried out as a clinical procedure with the intent of achieving a hard root surface since the roots of all teeth are relatively soft through the entire cementum and into the dentin to an average depth of 100 microns. Thus, the long standing clinical concept that the "soft" outer cementum must be planed away needs further evaluation.

All cementum is softer than calculus, enamel, and central crown and root dentin. However, the hardness of cementum is apparently not changed prior to a disease process as proposed by Gottlieb,[34] or following exposure to a periodontal pocket as thought by many clinicians since exposed and unexposed cementum are of equal hardness.

SUMMARY

1. No difference was found in the hardness of cementum which was exposed to a periodontal pocket compared to unexposed cementum.

2. Cementum was found to have an average hardness of 40 KHN compared to central crown and root dentin of 69 KHN.

3. No difference was observed in hardness between inner and outer cementum.

4. A narrow peripheral band of dentin underneath cementum had a comparable hardness to cementum and was much softer than the adjacent central dentin.

5. Peripheral dentin underneath enamel was softer than the central coronal dentin.

6. There was an immediate and dramatic change in hardness at the cemento-enamel junction with peripheral dentin covered by enamel being harder than peripheral dentin covered by cementum.

7. Central crown dentin had the same hardness as central root dentin.

8. Peripheral dentin near the pulp was softer than central root and crown dentin.

9. Transparent root dentin, as it occurred in the apical one-third of roots of some teeth, was softer than central root and crown dentin.

10. Preliminary measurements indicated that calculus was much harder than cementum and somewhat harder than dentin.

REFERENCES

1. Emslie, R. D., and Stack, M. V. The microhardness of roots of teeth with periodontal disease. Dent. Pract. and Dent. Rec., 9:101-13, Dec. 1958.

2. Ichiro, Nihei. A study of the hardness of human teeth. Osaka Univ. Dent. Soc. J., 4:1-20, Jan. 1959.

3. Box, H. K. Treatment of the periodontal pocket. Toronto, University Toronto Press, 1928. 123 p. (p. 91-101).

4. Glickman, Irving. Clinical periodontology.

2nd ed., Philadelphia, Saunders, 1958. 977 p. (p. 217).

5. Goldman, H. M. A rationale for the treatment of the infrabony pocket: one method of treatment, subgingival curettage. J. Periodont., 20:83-91, July 1949.

6. Orban, B., and Manella, V. B. A macroscopic and microscopic study of instruments designed for root planing. J. Periodont., 27:120-36, Apr. 1956.

7. Prichard, John. The intrabony technic as a predictable procedure. J. Periodont., 28:202-16, Jan. 1957.

8. Ramfjord, Sigurd. Reattachment in periodontal therapy. Am. Dent. A. J., 45:513-23, Nov. 1952.

9. Riffle, A. B. The cementum during curettage. J. Periodont., 23:170-7, July 1952.

10. Riffle, A. B. The dentin: its physical characteristics during curettage. J. Periodont., 24:232-41, Oct. 1953.

11. Schaffer, E. M. Histological results of root curettage of human teeth. J. Periodont., 27:296-300, Oct. 1956.

12. Schaffer, E. M. The new attachment operation in subcrestal pockets. Oral Surg., Oral Med., and Oral Path., 11:253-65, Mar. 1958.

13. Schaffer, E. M., and Zander, H. A. Histologic evidence of reattachment of periodontal pockets. Paradontologie, 3:101-6, Oct. 1953.

14. Riffle, A. B. The cervical enamel: its physical characteristics during curettage. J. Periodont., 22:225-33, Oct. 1951.

15. Riffle, A. B. The cemento-enamel junction. J. Pieriodont., 23:41-7, Jan. 1952.

16. Raper, H. R. Radiodontia in operative dentistry. Am. Dent. A. J., 18:716-32, Apr. 1931.

17. Maden, B. S., and Brudevold, Finn. Study of cervical radiolucent areas in human teeth. J. Periodont., 23:90-5, Apr. 1952.

18. Röchert, Hans. A quantitative x-ray microscopal study of calcium in the cementum of teeth. Acta Odont. Scand., 16 Suppl. 25, 1958.

19. Stones, H. H. Oral and dental disease. 3rd ed., Edinburg, Livingstone, 1954. XIX + 1019 p. (p. 451-6).

20. Bartelstone, H. J. Survey of the use of radioactive isotopes in dentistry. N.Y.S. Dent. J., 20:322-36, Aug.-Sept. 1950.

21. Wainwright, W. W. The permeability of human dental root structures to radioactive iodine. J. Periodont., 23:95-102, Apr. 1952.

22. Kronfeld, Rudolf. The biology of cementum. Am. Dent. A. J. and Dent. Cosmos, 25:1451-61, Sept. 1938.

23. Thoma, K. H., and Goldman, H. M. The pathology of dental cementum. Am. Dent. A. J., 26:1943-53, Dec. 1939.

24. Henry, J. L., and Weinmann, J. P. The pattern and repair of human cementum. Am. Dent. A. J., 42:270-90, Mar. 1951.

25. Harvey, B. L. C., and Zander, H. H. Root surface resorption of periodontally diseased teeth. Oral Surg., Oral Med., and Oral Path., 12:1439-43, Dec. 1959.

26. Zander, H. A., and Hürzeler, B. Continuous cementum apposition. J. Dent. Res., 37:1035-44, Nov.-Dec. 1958.

27. Hodge, H. C., and McKay, H. The microhardness of teeth. Am. Dent. A. J., 20:227-33, Feb. 1933.

28. Wright, H. N., and Fenske, E. L. Factors involved in variability in hardness of tooth structures. J. Dent. Res., 17:297, Aug. 1938.

29. Craig, R. G., and Peyton, F. A. The microhardness of enamel and dentin. J. Dent. Res., 37:661-8, Aug. 1958.

30. Craig, R. G., Gehring, P. E., and Peyton, F. A. Relation of structure to the microhardness of human dentin. J. Dent. Res., 38:624-30, May-June 1959.

31. Martens, P. J., Bradford, C. W., and Frank, A. M. Tissue changes in dentin. Int. Dent. J., 9:330-48, Sept. 1959.

32. Nalbandin, John. Age changes in human teeth. J. Dent. Res., 38:681, July-Aug. 1959 (Abstr.).

33. Kotanyi, Emmerick, Cementum. p. 171. (In Orban, H. J., ed. Oral Histology and Embryology, 4th ed., St. Louis, Mosby, 1957. 379 p.).

34. Gottlieb, Bernhard. Biology of cementum. J. Periodont., 13:13-17, Jan. 1942.

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Comparative Study of Saline Packs and Astringent Packs in Reducing Depth of Periodontal Pockets*

by JAMES L. PADGETT, D.D.S., M.S.D., *Monroe, Louisiana*

THE application of medicaments to the gingival tissue for treatment of periodontal disease has been advocated for many years. A review of the literature shows that three types of packing material have been used, paraffin wax base, powder and liquid mix, cotton twists saturated with medicinal agents.

In 1933, Box[1] discussed the use of "Dunlop wax" in treatment of periodontal pockets. Softened wax was injected into the pockets not exceeding 4.0 mm. and allowed to remain two to three days. The wax was then removed and scaling procedures performed. The advantages for this procedure were cited; reduction of the edematous hyperplastic tissue by pressure, and improved access into the periodontal pockets for scaling purposes by distention of the pocket wall due to packing.

The powder and liquid type of pack is used as an important adjunct in conservative periodontal procedures and as a post surgical dressing. Many variations of formulae[2, 3] can be found. Basically the powder is a mixture of zinc oxide and rosin and the liquid is composed of eugenol and a bland oil. The technique and advantage of this type of pack when used in treatment of edematous hyperplastic gingivitis are essentially the same as described in the use of paraffin wax packs.

The third type of pack, cotton twists saturated with medicinal agents, differs somewhat from the other two types in the therapeutics involved. This pack not only employs the use of pressure but also used the chemical action of drugs in reducing edematous hyperplastic gingivitis. Clinicians[4, 5, 6] listed various drugs used that may be escharotic or astringent, depending on the drug concentration. A few such drugs are zinc chloride, phenol, trichloroacetic acid, iodine and glycerine, silver nitrate and tannic acid.

Lundquist[7] described a method of treating edematous hyperplastic gingivitis that involved scaling the teeth and placing of zinc chloride packs and supportive massage. After scaling the teeth, a few strands of cotton dipped into an 8% aqueous solution of zinc chloride were packed into the periodontal pocket completely around the teeth until the tissues blanched and became anemic. The packs were allowed to remain in place for 15 to 20 minutes. The process was repeated at 10 day intervals for two subsequent appointments. After the third sitting, the scaling and packing procedures would have removed the irritational causes of gingivitis and the previously edematous gingiva would have been reduced to its normal contour. Lundquist explained that it was the mechanical pressure of the pack together with the astringent action of the drug that brought about changes in the inflamed tissue. He gave support to the action of mechanical pressure by citing Menkin,[8] Drinker and Yoffey.[9] He said that inflammation was characterized by capillary changes; that is, dilation and deformation of the vessels and an extravascular infiltration of cellular elements and blood proteins into the inflamed tissues. To return such elements to the blood vessels via the lymphatics an outside force was necessary. This could be accomplished by pressure packs and massage.

The zinc chloride element of the pack described by Lundquist[7] contributes a chemical astringent action on the inflamed tissue. The salts of zinc, principally zinc sulfate and zinc chloride, may act as astringents, mild antiseptics or corrosives. They owe their action to the ability of the zinc ion to precipitate protein. According to Sollman,[10] metallic salts such as zinc chloride combine with protein material to form more or less insoluble compounds. When the precipitation compound is insoluble, penetration into the tissue is not deep and astringent action results. Harrow and Mazur[11] stated that proteins are precipitated by the salts of heavy metals such as zinc chloride to form zinc proteinate.

Dobbs[12] explained that when zinc chloride is applied to the mucous membrane it forms a thin skin-like protective film which is composed of the precipitated protein material from fibrogenous and albuminous substances. Dobbs also explained that the astringent action of zinc chloride manifests itself in a combination of four definite ways, by contracting the muscular coat of small vessels, by condensing connective tissue, by diminishing secretion and exudation, and by checking migration of leukocytes. He related these actions to the ability of the zinc ion to precipitate protein. Diminishing of secretion, exudation and leukocytic infiltration results from precipitation of intercellular protein. Such a precipitation may occur among endothelial cells or in the ground substance which block the escape of blood serum and cells. Condensation within the connective tissue is achieved by reduction in the volume of protein component of connective tissue by formation of zinc proteinates.

Various solutions of zinc chloride can be used

*Summary of a thesis submitted in partial fulfillment of the requirements of Northwestern University for the degree of Master of Science In Dentistry. Degree conferred June 15, 1959. Research directed by Marjorie Houston, D.D.S., M.S.

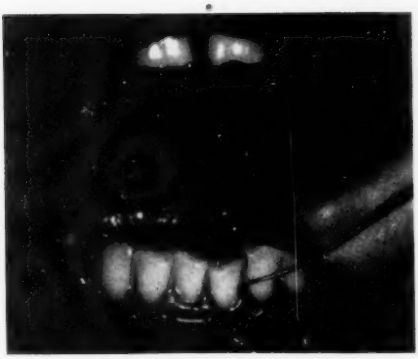


Fig. 1. Marking the location of the gingival crest.

in oral therapeutics. A 1:3000 aqueous solution is recommended for continuous use as a mouth wash, while a 1:1000 solution is recommended for temporary use only. Lundquist used an 8% aqueous solution for medicinal packs applied to edematous gingiva. In preparing any of these solutions a minute amount of hydrochloric acid must be added. The presence of the acid prevents the hydrolysis of zinc chloride forming basic zinc chloride (ZnOHCL) or zinc oxychloride (Zn_2OCl_2) which are insoluble in aqueous solution.[13]

In addition to an astringent action, dilute solutions of metallic salts such as zinc chloride exert a mild antiseptic action. According to MacClintic,[14] zinc chloride is a poor germicide and a feeble antiseptic, though a fair deodorant in concentrations of 1:200 to 1:500. Zensser's[15] text classified zinc chloride as a weak protoplasmic poison. In this instance the precipitation action of the zinc ion was the effective mechanism. Dobbs[12] explained that some cellular proteins behave as enzymes or coenzymes in metabolic reactions necessary for the vitality of bacterial cells. Thus, any inactivation of these systems by protein precipitation would alter vital cell processes causing a resultant bacteriostasis.

The purpose of this investigation is to study and compare the action of an astringent pack against the action of a saline pack under the following conditions:

1. In cases of edematous hyperplastic gingivitis associated with upper and lower anterior teeth.

2. Using cotton twists saturated in 8% aqueous solution of zinc chloride as the astringent pack.

3. Using cotton twists saturated in sterile physiological saline solution as the saline pack.

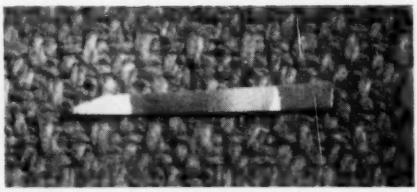


Fig. 2. Metal strip showing the location of the incisal edge, gingival crest, and the bottom of the pocket.

METHODS AND MATERIALS

Twenty-eight patients with edematous hyperplastic gingivitis were selected for this experiment. The areas of the experiment were confined to the anterior region of the upper and lower dental arches. The procedures performed on the selected areas were of the following types:

1. Treatment

a. Scaling of the teeth.

b. Placement of a cotton twist pack saturated in an 8% aqueous solution of zinc chloride into the periodontal pockets of the right side of the upper and lower arches (treated area) for twenty minutes. Note Figure 3.

c. Placement of a cotton twist pressure pack saturated in a physiological saline solution into the periodontal pockets of the left side of arches (control area) for twenty minutes.

2. Measurements: To observe the effect of treatment on the periodontal pockets, a tooth and its surrounding gingiva from the upper and lower treated area were chosen, as well as the corresponding teeth and tissues from the control side. Narrow nickel alloy strips were inserted into the periodontal pockets in order to determine pocket depth. At each visit measurements of the position of the gingival margin and the bottom of the periodontal pocket were recorded on the labial and one proximal aspect of the teeth chosen. A point on the incisal edge of the tooth was used as a relative point of measurement. Note Figure 1 and 2.

3. Radiographs: Radiographs of the teeth were taken with the metal strips against a proximal surface and extending to the bottom of the periodontal pocket. Note Figure 4.

The order of these procedures were as follows:

Visit 1
Scaling, Measurements, Radiographs.

Visits 2, 3, 4
Pack application and Measurements.

Visit 5
Measurements and Radiographs.

RESULTS AND DISCUSSION

The findings derived from the investigation can be considered in the following phases:

1. The change in the location of the crest of the gingival margin following a single scaling of the teeth and following a series of three gingival pack treatments.

2. The change in the location of the bottom of the pocket following a single scaling procedure and following a series of three gingival pack treatments.

3. The change in the depth of the periodontal pocket due to the combined effects of No. 1 and No. 2 above.

The following terms will be used:

Treat—refers to the tissues to which cotton packs saturated with 8% aqueous solution of zinc chloride had been applied.

Control—refers to the tissues to which cotton packs saturated with physiologic saline solution had been applied.

Favorable response—refers to either:

a. Apical shift in the location of the crest of the gingival margin.

b. A coronal shift in the location of the bottom of the pocket.

The following is a discussion of the results of a group of sixteen patients subjected to the procedures described.

Fig. 3. Zinc chloride packs in place causing blanching of the tissue.

The results are not fully significant until they are analyzed separately according to the procedures performed. The effect of a single scaling on the gingival margin is shown in Table IA, a to b. In these cases there was no significance in the terms "treat" and "control" since scaling procedures were attempted with equal thoroughness. It may be significant that in both labial and proximal aspects of the periodontal pockets there was significantly more apical shift in the crest of the gingival margin of the upper arch following a single scaling procedure than there was in the corresponding pockets of the lower arch. It is evident that scaling helps to reduce gingival inflammation by removing the cause of irritation. However, there should be some additional influence to ex-

Fig. 4. Radiograph of strip inserted to bottom of pocket.

TABLE I

		LABIAL				*PROXIMAL*			
A Location of the gingival margin.		*UC*	*LC*	*UT*	*LT*	*UC*	*LC*	*UT*	*LT*
	a to b.	+0.42	+0.27	+0.63	+0.28	+0.72	+0.31	+0.84	−0.11
	b to c.	+0.19	+0.29	+0.04	+0.14	+0.28	+0.23	+0.34	+0.36
+ indicates apical shift	c to d.	+0.11	+0.06	+0.06	+0.16	+0.13	+0.19	+0.16	+0.14
− indicates coronal shift	d to e.	+0.05	+0.08	+0.19	+0.17	+0.00	+0.16	+0.09	+0.17
	a to c.	+0.77	+0.70	+0.92	+0.75	+1.13	+0.89	+1.25	+0.56
	b to e.	+0.35	+0.43	+0.29	+0.47	+0.41	+0.58	+0.41	+0.67
B Location of bottom of pocket.									
	a to b.	−0.08	−0.06	−0.00	−0.05	+0.16	+0.03	+0.61	−0.13
	b to c.	−0.00	−0.02	−0.08	−0.06	−0.08	−0.13	+0.02	−0.17
− indicates coronal shift	c to d.	−0.03	−0.00	−0.05	+0.06	−0.20	−0.04	−0.08	+0.16
+ indicates apical shift	d to e.	+0.02	−0.02	+0.08	−0.03	−0.03	+0.22	+0.09	−0.09
	a to c.	−0.09	−0.09	−0.05	−0.08	+0.25	+0.08	+0.64	−0.23
	b to e.	−0.01	−0.03	−0.05	−0.03	+0.08	+0.05	+0.03	−0.10
C Average pocket depth before treatment.		2.47	2.64	2.64	2.92	3.42	3.97	3.53	3.73
D Average pocket depth after scaling.		1.97	2.31	2.01	2.59	2.86	3.69	3.30	3.70
E Average pocket depth after packs.		1.61	1.84	1.67	2.08	2.55	3.16	2.92	2.94
F Total reduction of pocket due to D and E.		0.86	0.79	0.97	0.83	0.88	0.81	0.61	0.79

a–first visit, b–second visit, c–third visit, d–fourth visit, UT–upper treat, LT–lower treat, UC–upper control, LC–lower control.

plain the consistent difference in the response to scaling in the upper and lower arches. One factor that is different is that the original depth of the periodontal pockets of the upper arch were less than those of the lower arch (Re Table IC). This indirectly indicates that the total amount of inflamed tissue is less in the upper arch and the processes that bring about resolution of the inflammation may be more effective in a shorter period of time in the upper arch than in the lower arch after a single scaling procedure. This action would be reflected by more apical shift in the location of the crest of the gingival margin in the upper arch than in the lower arch.

The changes that occurred in the location of the crest of the gingival margin, following the three visits when gingival packs were applied to the periodontal pockets, denote the influence that zinc chloride and pressure had on reducing inflammation. In both the labial and proximal aspects there was:

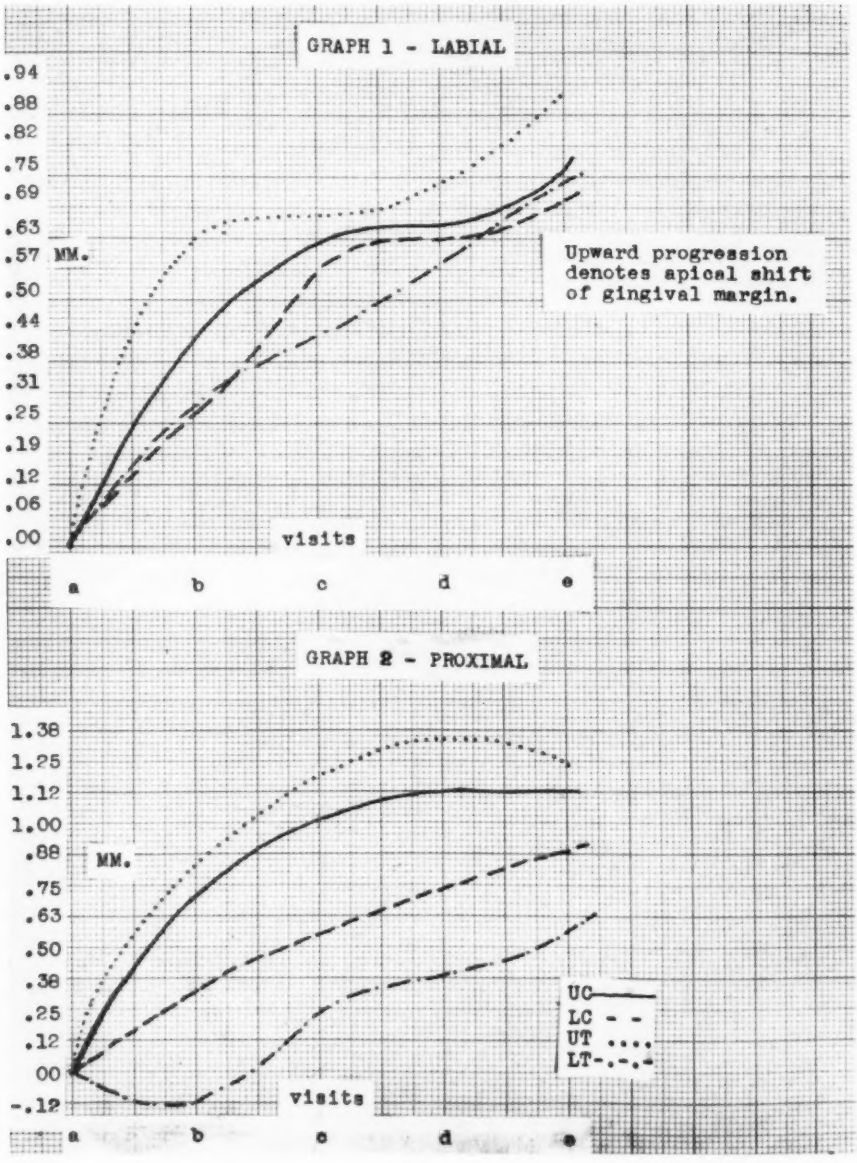
1. more favorable response in the lower treated area than the upper treated area.

2. slightly more favorable response in the treated area than the control area (Re Table IA, b to e; Table IB, b to e).

The significance of the difference in No. 1 above may be related to the amount of inflamed tissue involved. Periodontal pockets with more inflamed tissue have a greater potential to show more pocket reduction with adequate treatment over a prolonged period of time than pockets with less inflamed tissue. The same may be said by simply stating that deep periodontal pockets which respond favorably to treatment have the potential ability to show more actual apical shift in the location of the crest of the gingival margin than the more shallow pockets treated similarly. This explanation becomes logical when applied to the results in No. 1 above.

From No. 2 above, it can be concluded that in addition to the mechanical pressure of the pack, the astringent action of the zinc chloride produced a more favorable response than the packs containing physiologic saline solution.

By correlating Table IC with graphs 1 and 2 the relationship between pocket



a.—first visit.
b.—second visit.
c.—third visit.
d.—fourth visit.

UC—upper control.
LC—lower control.
UT—upper treat.
LT—lower treat.

TABLE II

	LABIAL				PROXIMAL			
	UC	LC	UT	LT	UC	LC	UT	LT
A % of apical shift of gingival margin due to scaling.	54.5	38.5	67.9	37.3	63.7	34.6	67.2	−19.7[1]
B % of apical shift of gingival margin due to packs.	45.5	61.5	31.2	62.7	36.3	65.4	32.8	80.3
C % of total apical shift due to A and B.	89.5	88.6	94.8	90.4	128.4[3]	109.9[3]	204.9[3]	70.9
D % of pocket reduction due to change of bottom of pocket.	10.5	11.4	5.2	9.6	−28.4[2]	−9.9[2]	−104.9[2]	29.1
E % of success based on establishment of 1.5 mm. sulcus.	88.7	69.3	76.3	58.5	45.9	32.8	30.0	35.4

[1]Negative percentile indicates coronal shift of gingival margin.

[2]Negative percentile indicates apical shift of bottom of pocket.

[3]Excess of one hundred indicates apical shift causing overall reduction of pocket depth despite result in D.

depth and favorable response becomes significant as influenced by scaling and a series of three gingival packs.

Another important consideration is the change that occurred in the location of the bottom of the pocket as influenced by a single scaling procedure and gingival packs. On the labial aspect the procedures of scaling and packing resulted in a slight coronal shift of the bottom of the pocket. The pockets on the labial were shallow and access for scaling procedures were favorable. Therefore, removal of calculus adherent to the tooth surface was thorough and consequently, the action of the packing could have allowed the coronal shift of the bottom of the pocket by (1) "reattachment" of the epithelial attachment apparatus in a coronal direction, (2) closer adaptation of the pocket wall by reduction of edema and tightening of gingival periodontal fibers, especially the circumferential fibers, thus giving the impression of a coronal shift in the location of the bottom of the pocket.

On the proximal aspect the procedures of scaling and packing resulted in a slight tendency toward an apical shift in the bottom of the pocket. Here the pockets were deeper and involved more inflamed tissue than on the labial aspect. It is possible that under such conditions scaling was not as thorough and also that the epithelial attachment at the bottom of the pocket was subjected more to separation from the tooth surface. If some minute inflammatory elements such as calculus remained localized at the bottom of the pocket, then the mechanical pressure of the pack may have acted adversely only at the bottom of the pocket resulting in a very slight apical shift of the location of the bottom of the pocket. It should be noted that, in any case, the amount of apical or coronal shift in the bottom of the pocket was very slight, usually not over 0.10 mm.

Table II expresses the relative influence of scaling (A); the application of packs (B) separately and in combination (C) in producing an apical shift in the location of the crest of the gingival margin. It also expresses the influence of both scaling and packs on the location of the bottom of the pocket (D). In (D) a negative percentile indicates an apical shift. In (C) a percentile in excess of 100% indicates an apical shift in the location of the crest of the gingival margin which not only compensated for the apical shift of the bottom of the pocket, but also resulted in an over-all reduction of the periodontal pocket. From this table it can be seen that all pockets treated were reduced. Table IIE shows that the greatest relative success of the total

treatment of both scaling and packing in establishment of a 1.5 mm. sulcus occurred in the periodontal pockets which were less than 3.0 mm. These findings indicate that when the pocket depth is from 3.0 mm. to 5.0 mm. reduction of the pocket to 1.5 mm. or less tend to be less than 50% successful.

Radiographic comparison of the change in the location of the bottom of the pocket, before and after treatment, could not be evaluated with the necessary critical observation. The factors of minute measurements involved and experimental error negated the significance of relating two sets of radiographs of the same object to a common entity.

SUMMARY

After tabulation and analysis of the findings the following *conclusions* were made:

1. Both scaling procedures and application of pressure and astringent packs played a part in reducing the depth of the periodontal pocket in edematous hyperplastic gingivitis of the anterior region in the upper and lower dental arches. The role of each in shifting the crest of the gingival margin in an apical direction can be listed:

a. A *single* scaling procedure was more effective in causing an apical shift in the crest of the gingival margin of shallow pockets than of deeper pockets in the same relative area.

b. A series of treatments using either pressure packs containing saline, or astringent packs containing zinc chloride, resulted in more actual apical shift of the crest of the gingival margin in moderately deep periodontal pockets rather than in more shallow pockets of the same area.

c. A cotton astringent pack containing 8% aqueous solution of zinc chloride was slightly more effective in causing an apical shift in the crest of the gingival margin than a cotton pressure pack containing physiologic saline solution in any area of the anterior region of the dental arches.

2. Scaling procedures and application of pressure packs, regardless of the astringent nature, caused a slight shift (not more than an average of 0.18 mm.) in the location of the bottom of the periodontal pocket in the following manner:

a. Scaling and packing procedures tended to cause a slight coronal shift in the bottom of shallow pockets where access into the pocket was favorable.

b. Scaling and packing procedures in deep pockets where access was not favorable resulted in a slight apical shift of the bottom of the pocket.

3. Treatment of all pockets resulted in reduction of pocket depth as follows:

a. Those cases which showed a coronal shift in the bottom of the pocket also showed an apical shift in the crest of the gingival margin.

b. Those cases which showed an apical shift in the bottom of the pocket also showed adequate apical shift of the crest of the gingival margin to compensate for the unfavorable change of the location of the bottom of the pocket.

4. Radiographic evidence of change in the location of the bottom of the pocket after five weeks of treatment is extremely difficult to ascertain.

The Effect of Highly Concentrated Solutions of Stannous Fluoride on Human Gingival Tissue

by Robert P. Swieterman, Joseph C. Muhler, and Henry M. Swenson,
Indianapolis, Indiana

THE use of topical fluoride treatments as an anticariogenic agent has greatly increased during the past two years.* This is a result of many factors, but probably one of the most important is the greater utility of stannous fluoride over sodium fluoride. For example, while sodium fluoride was effective only in children, stannous fluoride has been found to be effective in adults[1] as well as children whose teeth have calcified while living in an optimal fluoride area.[2] Of even greater importance, however, is that fact that stannous fluoride involves only a single treatment in contrast to the four treatments required when sodium fluoride is used.[3] Due to the increased utility of stannous fluoride and its frequent use in adults, more attention should be given to the effect of stannous fluoride on gingival tissue with various types of pathology. No information is available concerning this subject except the work of Muhler[4] in which it was reported that in about 5 per cent of the cases treated with either an 8% or 10% solution of stannous fluoride, the peripheral portion of the interdental papilla was blanched if a gingivitis was present at the time of the topical application. Lal[5] studied the gingiva after topical application of sodium fluoride or sodium chloride, and reported a slight shrinkage of the superficial layers of epithelium, but histologic evaluation revealed no appreciable changes.

This study was supported in part by Grant No. D-1142 from the Department of Health, Education, and Welfare in Indiana University, School of Dentistry, Indianapolis, Indiana.

*Accurate estimates of the use of stannous fluoride are available since Indiana University distributes, gratis, samples of stannous fluoride to dentists for use in their preventive dentistry programs. During 1959, for example, more than 15,000 samples were distributed from this source alone. It is estimated that 25% of the dentists in the United States and 45% of the dentists in Canada are currently using stannous fluoride as an adjunct to their preventive dentistry programs.

METHOD AND MATERIALS

Clinically evaluated normal and periodontally involved gingival soft tissues were obtained from patients at the clinic of the Indiana University Dental School. The patients were of both sexes, ranging in ages from 18 to 69 years, and were equally divided between both the negro and caucasian races. Clinical and radiographic examination in addition to a critical evaluation of the gingival soft tissue was made of each case and a diagnosis of the periodontal condition recorded. The various types of periodontal cases used in this study included gingivitis, periodontitis, periodontosis, and a single case of dilantin hyperplasia.

A thorough dental prophylaxis was performed on each patient and Kodachrome photographs were taken so as to have permanent records for study. Application of either stannous fluoride, stannous chloride, sodium fluoride, or sodium chloride, at concentrations ranging from 2 to 20 per cent were applied immediately. The technique of topical application was identical to that described by Muhler,[6] except the soft tissue, instead of the teeth, were treated with the respective solutions. The solutions were applied with a sterile cotton applicator.

After lightly drying the treated areas with warm air, a clinical examination was made of the gingiva and a second Kodachrome photograph taken. The treated areas were surgically removed with gingivectomy knives and the tissue placed in 10 per cent formalin. Tissue removals were accomplished at intervals of one to twenty-eight days following topical applications.

A control group of ten patients whose

TABLE I
Effects of 2% and 4% NaF, and 15% NaCl on Periodontally Involved Gingival Soft Tissues

Age of Patient (yrs)	% of Solution	Compound	Area	Tissue Removed	Degree of Inflammation Present at the Time of Topical Application	Reaction on Gingiva	Type of Epithelium	Depth of Penetration	Diagnosis of Case
63	2	NaF	4-3	Immediate	mild	none	P	none	Periodontitis
49	2	NaF	4-1	Immediate	mild-moderate	none	P	none	Periodontitis
30	4	NaF	5-4	30 minutes	mild-moderate	none	K	none	Periodontitis
53	4	NaF	5-4	30 minutes	moderate	none	P	none	Periodontitis
52	15	NaCl	3-1	Immediate	mild-moderate	none	P	none	Gingivitis, mild
63	15	NaCl	5-3	30 minutes	mild	none	K	none	Periodontitis

P = parakeratin; K = keratin; S = spinous cell layer; ** = medium coating.

periodontal conditions were classified as normal, were also treated with stannous fluoride and included in this study. Of this group, four male patients with normal gingiva had tissue specimens removed at intervals of three, four, five, and six days after stannous fluoride application. Six patients had tissue removed at seven, fourteen, twenty-one, and twenty-eight day intervals.

All tissues were sectioned at 7 microns, and stained in Harris' hematoxylin and eosin, and mounted in Permount.*

DATA AND DISCUSSION

The data in this study are found in Tables 1 through 5 and Illustrations 1 through 17. The results using sodium fluoride and sodium chloride corroborate the conclusions reported by Lal,[5] in which no gross changes in the gingival tissues were reported. In contrast to the studies of Lal,[5] the cases selected for this study consisted of various types of degrees of periodontal disease. Table 1 shows the effect of either a 2 or 4 per cent solution of sodium fluoride or a 15 per cent sodium chloride solution on the two types of gingival diseased tissues. Neither of these compounds produced any gross gingival changes. In contrast to these findings, the results in Tables 2 and 3 show that both stannous fluoride and stannous chloride result in gingival change ranging in severity from slight to heavy. These gingival changes are a function of the degree of gingival inflammation at the time the solution was applied. Tables 4 and 5 show that the use of a 20 per cent solution of stannous fluoride applied to normal gingival tissue does not result in any noticeable gingival changes.

*Fisher Scientific Company, Fair Lawn, New Jersey.

TABLE II
Effects of 10% SnF_2 or 10 and 20% $SnCl_2$ on Periodontally Involved Gingival Soft Tissue

Age of Patient (yrs)	% of solution	Compound	Area	Tissue Removed	Degree of Inflammation Present at the Time of Topical Application	Reaction on Gingiva	Type of Epithelium	Depth of Penetration	Diagnosis of Case
47	10	SnF_2	3-2	Immediate	moderate	medium	P	P	Periodontitis
40	10	SnF_2	3-4	Immediate	mild	heavy	P	P	Periodontitis
18	10	SnF_2	3-5	Immediate	moderate	slight	K	K & S**	Periodontosis
63	10	SnF_2	2-5	1 hour	mild	slight	P	P	Periodontitis
28	10	SnF_2	3-1	1 day	severe	heavy	P	none	Gingivitis
36	10	SnF_2	3-1	2 days	severe	slight	P	P	Periodontitis
53	10	SnF_2	1-3	1 week	moderate	slight	K	K	Periodontitis
53	10	SnF_2	3-1	1 week	mild	slight	P	P	Periodontitis
37	20	$SnCl_2$	2-1	Immediate	severe	medium	P	P	Periodontitis
38	20	$SnCl_2$	8	Immediate	mild	heavy	K	K & S	tooth extracted
43	10	$SnCl_2$	8	30 minutes	mild	heavy	K	K	tooth extracted
69	20	$SnCl_2$	4-1	30 minutes	mild	slight	P	P**	Periodontitis

P = parakeratin; K = keratin; S = spinous cell layer; ** = medium coating.

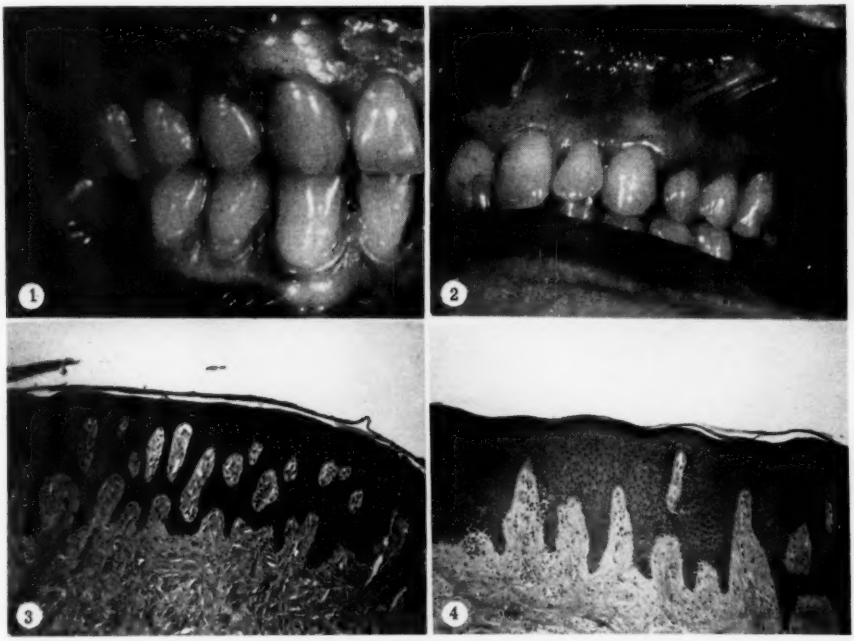


Figure 1
1. Immediately following topical application of 4% sodium fluoride solution to pathologic tissue. 2. Immediately following topical application of 15% sodium chloride solution to pathologic tissue. 3. Photomicrograph (10X) of gingival tissue immediately following topical application of 4% sodium fluoride solution to pathologic tissue. 4. Photomicrograph (10X) of gingival tissue immediately following topical application of 15% sodium chloride solution to pathologic tissue.

Illustration 1 shows the clinical effects of sodium fluoride and Illustration 2 the effects of sodium chloride on gingival tissue. Shrinkage of epithelial layers as reported by Lal[5] was not observed. Illustration 3 shows the histologic effect of sodium fluoride on gingival tissue. There are no morphological nor histologic changes in the epithelium. Illustration 4 shows the effects of sodium chloride on the epithelium. No morphological changes are noted.

Those patients who received topical applications of either stannous fluoride or stannous chloride all demonstrated grossly a coating of the gingiva (Illustrations 5 and 6) principally at the gingival crest if a gingival inflammation was present at the time of the topical treatment. This disappeared, however, within 24 hours in all cases. This coating varied in quantity and severity with the amount of inflammation clinically present at the time of the topical application. It was white, shiny, and smooth in appearance. No ulceration nor sloughing of tissue was visible. All areas disappeared without incident or discomfort to the patient. It is of interest to note that one case of periodontosis which was included in this study did not show any coating after application of stannous fluoride. The reason for this is not known.

The application of 10 per cent stannous chloride (Illustration 7) shows the white coating located in the lower right cuspid area at the gingival margin in a patient with gingivitis.

Illustration 8 demonstrates the effect of a 20 per cent stannous fluoride solution in a patient with moderately inflammed gingival tissue. There was no significant differ-

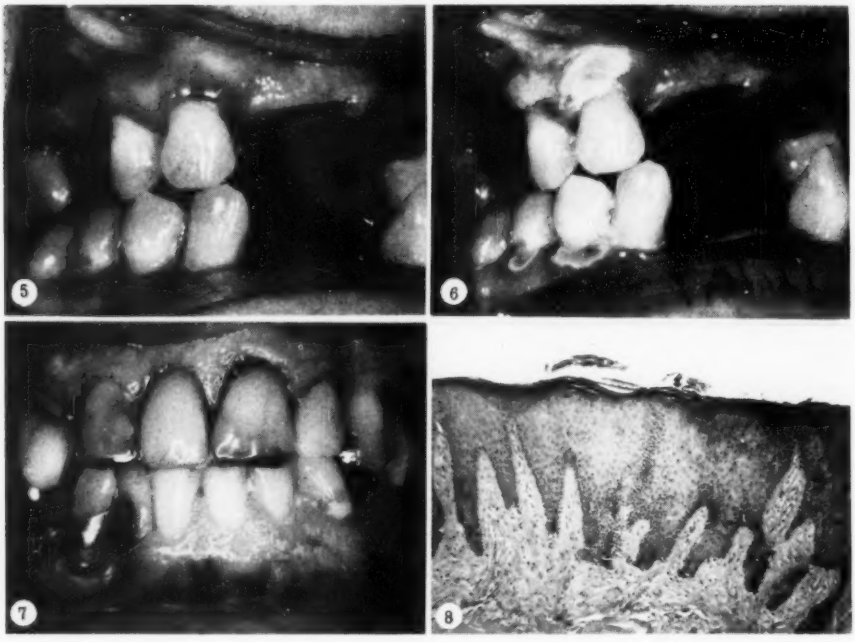


Figure 2
5. Inflamed gingival tissue, periodontitis before topical application. 6. Case illustrated in No. 5, immediately following topical application of 20% stannous fluoride solution. 7. Immediately following topical application of 10% stannous chloride, gingivitis. 8. Photomicrograph (10X) moderately inflamed gingival tissue, immediately following application of 20% stannous fluoride solution.

ence in the degree of gingival reaction between the 10 per cent or 20 per cent stannous fluoride solution.

It is of interest to note that the gingival reaction present seemingly is due to the influence of the stannous ion rather than the fluoride. This is not to be unexpected for stannous ion is a heavy metal belonging to a group of compounds long noted for their desquamatory effect on gingival tissue. Undoubtedly, this is a result of the heavy metal precipitating a portion of the protein in the less viable surface areas of the gingival tissue. However, other heavy metals may act also through a systemic mechanism by being absorbed by the blood with the anachoretic effect occurring at sites of inflammation in the oral cavity. Apparently stannous ion, as used in this study, does not act systemically, and the effect on the gingiva is strictly a local phenomenon.

Sulphides are known to react with metals to produce a metal sulphide. Since there are bacteria which produce sulphides in the oral cavity, plus the fact that the dietary contains many sulphide containing foods (eggs, corn, beef, cheese), these facts may contribute to the coating observed on the gingiva.

Illustration 9 demonstrates the clinical effect of 10 per cent stannous fluoride on normal gingival tissue. The effect of 20 per cent stannous fluoride on normal gingiva (Illustration 10) is shown by the white coating observed in the upper right cuspid-bicuspid area.

Shapiro et al.[7] reported on a single papillary biopsy following the topical applica-

TABLE III
Effects of 20% SnF_2 on Periodontally Involved Gingival Soft Tissues

Age of Patient (yrs)	*Area*	*Tissue Removed*	*Degree of Inflammation Present at the Time of Topical Application*	*Reaction on Gingiva*	*Type of Epithelium*	*Depth of Penetration*	*Diagnosis of Case*
32	4 3	Immediate	moderate	slight	P	P	Periodontitis
40	4 3	Immediate	severe	heavy	P	P & S	Periodontitis
29	1 2	Immediate	severe	heavy	P	P & S	Periodontitis
18	6 4	Immediate	moderate	no coating	K	K & S	Periodontosis
37	1 2	Immediate	moderate	medium	K	K & S	Periodontitis
44	2 1	Immediate	mild-moderate	heavy	P	P & S	Periodontitis
57	6 3	Immediate	severe	slight	P	P	Periodontitis
42	4 3	Immediate	mild	heavy	P	P & S	Periodontitis
12	6	30 minutes	moderate	slight	K	K	tooth extracted
36	3 1	30 minutes	moderate	slight	K	K**	Periodontitis
36	1 3	30 minutes	moderate	slight	K	K	Periodontitis
18	3 5	30 minutes	moderate	slight to unnoticeable	P	P & S	Periodontosis
32	6 3						
32	6 3	30 minutes	severe	heavy	P	P & S	Periodontitis
30	6 3	5 hours	mild	slight	P	P**	Periodontitis
38	6 3	1 day	severe	heavy	K	K	Dilatin Hyperplasia
40	5 3	1 week	severe	heavy	K	K**	Periodontitis
32	4 6	1 week	moderate	heavy	K	K	Periodontitis
28	3 1	1 week	severe	heavy	K	K	Periodontitis
38	5 3	1 week	moderate	medium	P	P**	Dilatin Hyperplasia
38	4 5	2 weeks	severe	heavy	P	none	Dilatin Hyperplasia
46	3 4	2 weeks	mild	slight	K	K**	Periodontitis
38	3 4	3 weeks	mild	heavy	K	K	Dilatin Hyperplasia

P = parakeratin; K = keratin; S = spinous cell layer; ** = medium coating.

TABLE IV
Normal Tissue, 20% SnF_2 Topically Applied

Case No.	*Age of Patient yrs*	*Area*	*Tissue Removed*	*Reaction of Compound*	*Degree of Inflammation*	*Type of Epithelium*	*Depth of Penetration*
1	38	3 2	1 week	slight	mild	keratin	K—
		4 2	2 weeks	heavy	mild	keratin	K—
		2 3	3 weeks	slight	mild	parakeratin	P—
		2 3	4 weeks	medium	severe	keratin	none
2	30	4 3	1 week	medium	mild-moderate	parakeratin	P—
		4 3	2 weeks	slight	mild	parakeratin	P—
		3 4	3 weeks	medium	moderate	keratin	K—
		3 4	4 weeks	slight	mild	keratin	K—
3	32	4 3	immediate	medium	mild	keratin	K & S
		4 3	1 week	slight	mild-moderate	keratin	K
		3 5	3 weeks	slight	mild	keratin	K & S
		3 4	4 weeks	slight	tissue lost in process	tissue lost in process	
4	32	4 2	1 week	medium	moderate	keratin	K
		4 3	2 weeks	slight	moderate	keratin	K
		3 4	3 weeks	slight	mild	parakeratin	none
		3 4	4 weeks	slight	moderate	keratin	none
5	38	5 4	1 week	slight	moderate	keratin	P—
		5 3	2 weeks	slight	mild	parakeratin	P—
		3 4	3 weeks	slight	moderate	parakeratin	P—
		4 5	4 weeks	slight	mild	keratin	K—
6	44	4 3	1 week	slight	mild	keratin	K—
		3 4	2 weeks	heavy	moderate	keratin	K—
		4 3	3 weeks	slight	mild	keratin	none
		3 4	4 weeks	slight	mild	parakeratin	none

P = parakeratin; K = keratin; S = spinous cell layer; — = very slight; — = slight.

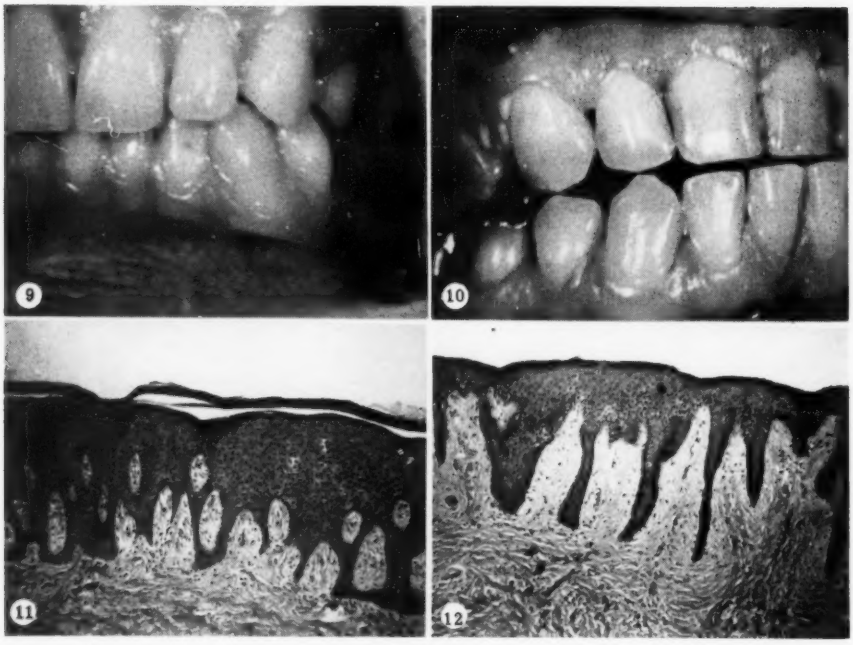


Figure 3
9. Immediately following topical application of 10% stannous fluoride solution to normal tissue. 10. Immediately following topical application of 20% stannous fluoride solution to normal gingival tissue. 11. Photomicrograph (10X) of pathologic gingival tissue immediately following topical application of 10% stannous chloride solution. 12. Photomicrograph (10X) of normal gingival tissue immediately following application of 10% stannous fluoride solution.

TABLE V
Normal Tissue, 20% SnF_2 Topically Applied

Case No.	*Age of Patient (yrs)*	*Area*	*Tissue Removed*	*Reaction of Compound*	*Degree of Inflammation*	*Type of Epithelium*	*Depth of Penetration*
7	26	$\frac{4\ 3}{}$	3rd day	°0	±	P	P & S
		$\frac{4\ 3}{}$	4th day	°0	±	P	P
		$\frac{3\ 4}{}$	5th day	°0	±	P	P
		$\frac{3\ 4}{}$	6th day	°0	±	P	P
8	24	$\frac{4\ 3}{}$	3rd day	°	+ +	K	K
		$\frac{4\ 3}{}$	4th day	°	+ + +	P	P
		$\frac{3\ 4}{}$	5th day	°	+	K	K
		$\frac{3\ 4}{}$	6th day	°	+	P	P
9	22	$\frac{4\ 3}{}$	3rd day	°0	+	K	K
		$\frac{4\ 3}{}$	4th day	°0	+ + +	P	P
		$\frac{3\ 4}{}$	5th day	°0	+	K	K
		$\frac{3\ 4}{}$	6th day	°0	+	K	K
10	28	$\frac{3\ 2}{}$	3rd day	0	+ + + +	P	P
		$\frac{4\ 3}{}$	4th day	0	+ + + +	K	K
		$\frac{2\ 3}{}$	5th day	0	+	K	K
		$\frac{3\ 4}{}$	6th day	0	+ +	K	K

0 = no coating
0° = slight to unnoticeable
° = slight coating
= medium coating
= heavy coating

P = parakeratin
K = keratin
S = spinous cell layer
+ = mild
+ + = mild moderate
+ + + = moderate
+ + + + = severe
− = very slight
− − = slight

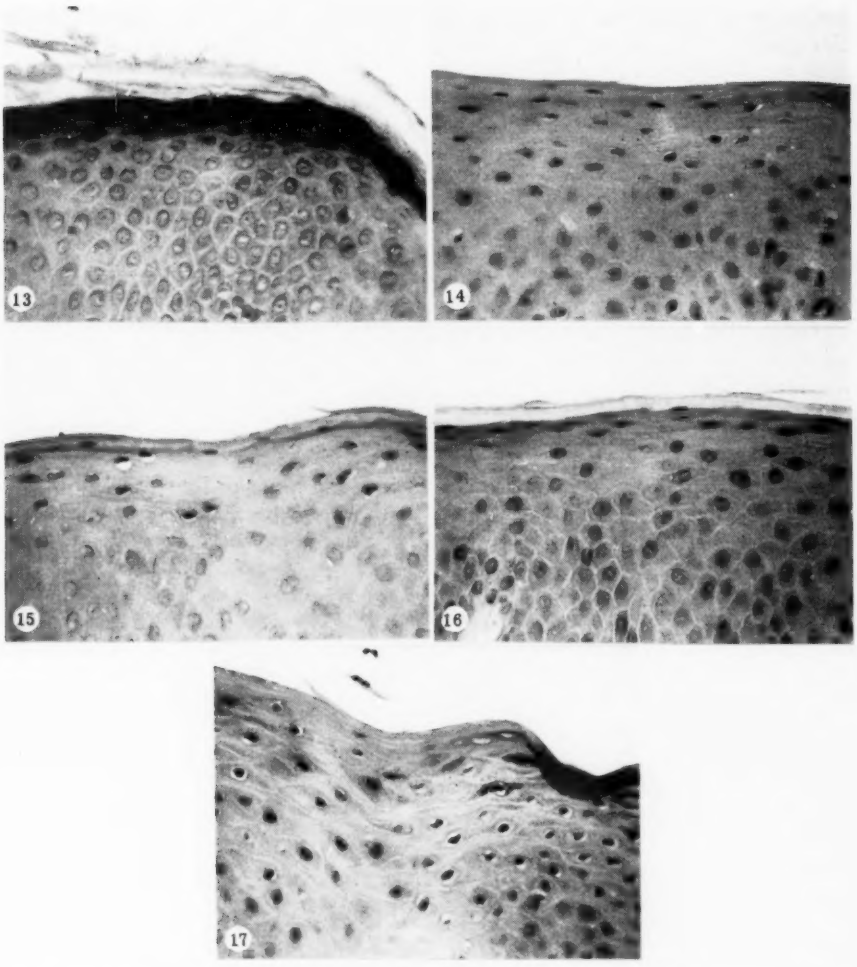


Figure 4
13. Photomicrograph (43X) normal tissue one week following application of 20% stannous fluoride solution. 14. Photomicrograph (43X) normal tissue, two weeks following application of 20% stannous fluoride solution. 15. Photomicrograph (43X) normal tissue, three weeks following application of 20% stannous fluoride solution. 16. Photomicrograph (43X) normal tissue, four weeks after application of 20% stannous fluoride solution. 17. Photomicrograph (43X) normal tissue, third day following application of 20% stannous fluoride solution.

tion of 0.4 per cent stannous fluoride in which deeply staining granular nuclei were found. In this study such a finding was not observed. However, a deeper staining of the superficial epithelial layer was noted. Illustration 11 shows deep staining of the keratin layer after the application of 10 per cent stannous fluoride solution to a patient with normal gingiva. Illustration 12 demonstrates a deeper staining which was limited to the parakeratin layer in a patient with normal gingiva who received a topical application of a 10 per cent solution of stannous fluoride.

The age of the patient may be a very important factor to evaluate in such

studies as these. An interesting aspect to this problem is a group of older patients with so-called normal gingiva whose soft tissues were treated with 20 per cent stannous fluoride and biopsied at 1, 2, 3, and 4 week intervals (Illustrations 13-16). They all showed a greater amount of tissue penetration as compared to the slight penetration into the epithelium as observed in the younger age group. Figure 17 shows only the keratin layer is slightly involved in the normal tissue of a young patient which was removed three days following the topical application of 20 per cent stannous fluoride solution.

SUMMARY

Patients with either normal or pathologically affected gingival tissues received topical applications of sodium chloride, sodium fluoride, stannous chloride, and stannous fluoride at concentrations ranging from 2 to 20 per cent. Gingival tissues were surgically removed immediately and in periods ranging to 28 days.

Gingival tissue treated with sodium chloride or sodium fluoride showed no gross or histologic changes when compared to untreated gingiva.

Patients who received stannous chloride or stannous fluoride all demonstrated a white, shiny, smooth coating of the gingiva, principally at the gingival crest. The concentration of stannous fluoride appeared to have no correlation with the amount of gross coating produced on the gingiva.

From this study no apparent harmful effects to the gingival tissues were observed from the use of stannous fluoride solutions up to and including concentrations of 20 per cent. This coating disappeared within 24 hours without incident. Clinical response of the gingiva to 20 per cent stannous fluoride solutions varied in intensity with the amount of inflammation present at the time of the initial treatment. Histologically, there was noted a deeper staining of the superficial epithelium which also was proportionate to the amount of inflammatory cells present in the subjacent connective tissue at the time of the initial treatment. Cell morphology was not changed by either the stannous fluoride or stannous chloride.

1121 W. Michigan Street

REFERENCES

1. Muhler, J. C.: The Effect of a Single Topical Application of Stannous Fluoride on the Incidence of Dental Caries in Adults. J. Dent. Res., 37:415, 1958.

2. Muhler, J. C.: The Anticariogenic Effectiveness of a Single Application of Stannous Fluoride in Children Residing in an Optimal Communal Fluoride Area. Results at the end of 30 months. J.A.D.A., 61:431, 1960.

3. Gish, C. W., Muhler, J. C., and Howell, C. L.: A New Approach to the Topical Application of Fluorides in Children with results at the End of Four Years. J. Dent. Child., 26:300, 1959.

4. Muhler, J. C.: Effect on Gingiva and Occurrence of Pigmentation of Teeth Following the Topical Application of Stannous Fluoride or Stannous Chlorofluoride. J. Periodontology, 28:281, 1957.

5. Lal, D.: Histological Study of Gingival Tissue After Topical Application of Sodium Floride, J. All-India D. A., 23:4, 1951.

6. Muhler, J. C.: Single Topical Stannous Fluoride Application Technique. J. Dent. Child., 25: 306, 1958.

7. Shapiro, M. and Hood, J. L.: Discoloration of Gingiva and Teeth by Stannous Fluoride. J. Calif. State Dent. Assn., 32:174, 1956.

Factors that Influence the Acetylating Activity of Blood

1. Effect of Addition of Certain Metabolites and Physiologically Active Substances

by D. Subrahmanyam, R. Quentin Blackwell and L. S. Fosdick, *Chicago, Illinois*

INSOFAR as blood from people suffering from periodontal disease acetylates para-aminobenzoic acid (PABA) much more rapidly than does normal blood, it was thought of interest to investigate the factors that may influence the acetylating activity of blood.[1] The speed of acetylation of blood is governed primarily by the concentration and activity of the enzyme acetylase, by the presence of co-enzyme A (CoASH) and the acetyl group. Insofar as one high energy bond is required in the reaction a source of energy for the synthesis of CoASH actyl is also involved. The factors that influence the acetylating activity of liver have been quite intensively studied[2] in vivo by Hensel and extended by Harrow.[3] The effect of various substances with the use of rabbit liver as the enzyme source has been reported by Klein and Harris[4] and by Lipman.[5] Johnston[6] found that narcotics in general would inhibit liver acetylase. Blondheim[7] found that in human blood glucose had a stimulating effect. This paper deals with the effect of certain metabolically and physiologically active compounds.

MATERIALS AND METHODS

The materials and methods used for the determination of the acetylating activity of blood are described in a previous paper.[1] The effect of compounds such as the common mono- and di-saccharides, acetate, pyruvate, dihydroxy acetone, alpha-keto glutaric acid, nembutal, fluoride, isoniazid, cortisone acetate, and 9-fluorocortisone on the acetylating activity of blood was studied. In the case of steroids, their effect on the blood acetylating activity of adrenalectomized rats also was studied. Blood sugar determinations were made by the method of Folin and Wu.[8]

RESULTS

Glucose. The effect of raising the blood glucose level upon the *in vitro* PABA acetylation by rat blood was studied; the results are shown graphically in Figure 1. The addition of increasing amounts of glucose was accompanied by a sharp rise in PABA acetylation by rat blood. This effect suggests that the added glucose might be metabolizing in blood to provide energy, as well as acetate or a precursor of acetate to the acetylating system. Further, these results indicate that the endogenous blood glucose of subjects in the fasting state might be the source of energy for the normal acetylating activity of blood.

The loss of acetylating activity in blood after 24 hours incubation might be due in part to the absence of energy-producing

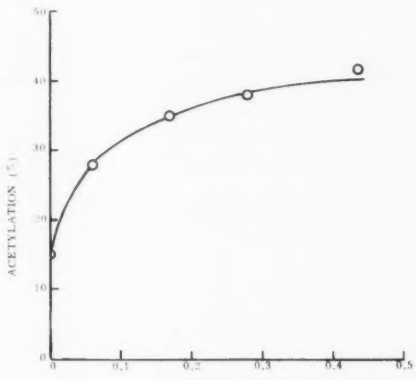


Figure 1

This study was supported by a United States Health Grant, No. D-395-C-1 in the Chemistry Department, Northwestern University Dental School.

This report is based on a dissertation submitted to the faculty of the Graduate School of Northwestern University by D. Subrahmanyam in partial fulfillment of the degree of Doctor of Philosophy.

TABLE I
Effect of Addition of Glucose to the Pre-incubated Human Blood on Its Acetylation Activity

Experiment Number	*% Acetylation*		
	No Glucose Added. Fresh Blood Incubated for 24 Hours	*(0.1 M) Glucose Added to 24 Hr. Pre-incubated Blood and Incubated for an Additional 24 Hr. Period*	*(0.1 M) Glucose Added to Fresh Blood and Incubated for 24 Hrs.*
1	23	39	52
2	25	40	51
3	27	42	50

glucose as well as to the inactivation of enzyme systems. Accordingly, the activity of the enzyme systems in incubated blood was tested by the following experiments.

PABA (0.2 ml. of the regular solution) was added to 5 ml. of human blood and the mixture incubated for 24 hours at 37°C. At the end of the incubation period, glucose, at a final concentration of 0.1 M, was added to the blood and the mixture was incubated for an additional 24 hour period. Control experiments with and without the addition of equal amounts of glucose to fresh blood were carried out simultaneously.

The results, given in Table 1, suggest that 24 hour pre-incubated blood still possessed acetylating activity because addition of glucose to the blood raised the acetylation values. However, the fact that fresh blood acetylated more than the pre-incubated blood in presence of same amount of added glucose indicates that partial inactivation of enzyme systems probably occurred during the first 24 hour incubation period.

Other Mono- and Di-Saccharides. The effect of other monosaccharides, fructose, galactose and the disaccharides, surose, lactose, at a final concentration of 0.1 M

TABLE II
Effect of Added Sugars on Acetylation by Rat Blood

Experiment Number	*% Acetylation in 24 hrs.*				
	No Sugar Added	*Glucose Added (0.1 M)*	*Fructose Added (0.1 M)*	*Fructose-6-Phosphate* Added (0.1 M)*	*Galactose Added (0.1 M)*
1	13	28	13	—	13
2	16	34	22	—	20
3	16	31	18	—	18
4	15	26	19	24	17
5	14	24	16	22	16
6	16	30	19	27	19

*4 gram of barium salt of fructose-6-phosphate were dissolved in 10 ml. of water and Ba^{++} was precipitated as the sulfate by the addition of 13 ml. of 0.5 M Na_2SO_4. The mixture was made up to 25 ml., centrifuged, and filtered. The clear filtrate was used in the experiments.

TABLE III
Effect of Added Sugars on Acetylation of Human Blood

Experiment Number	% Acetylation in 24 hrs.				
	No Sugar Added	Glucose Added (0.1 M)	Fructose Added (0.1 M)	Galactose Added (0.1 M)	Sucrose Added (0.1 M)
1	24	45	31	32	23
2	27	50	35	34	25
3	32	—	36	37	33

upon acetylation by rat and human blood was studied. The results are given in Table 2and 3. It is apparent from Table 2 that glucose and fructose-6-phosphate markedly increased acetylating activity, while fructose and galactose did not appreciably alter the normal values. Sucrose, maltose and lactose showed no appreciable influence upon the acetylating activity of rat blood. Glucose was the only sugar tried that materially increased acetylating activity in human blood.

ATP, CoASH and Sugar Metabolites. Because the increase in acetylation due to the added glucose is attributed partly to the liberation of energy, the effect of ATP, acetate and CoASH upon the acetylation of PABA by human blood was studied. The results indicate that these compounds alone or in combination did not appreciably alter the normal acetylation rate of human blood.

Addition of sugar metabolites such as alpha-ketoglutaric acid, dihydroxyacetone and pyruvate to rat blood did not show any significant effect on acetylation.

Fluoride. Fluoride is known to be a potent inhibitor of several enzymes; enolase, in particular, is inactivated at very low concentrations of this ion. It was reported[7] that fluoride inhibited the acetylation of PABA by human blood. Accordingly the effect of this ion on the acetylating activity and glucose metabolism in rat blood was studied; the results are presented in Table 4. Acetylation also was measured in blood samples containing fluoride with added glucose. Glucose did not cause an increase in the acetylation of PABA in the presence of fluoride, although the sugar did decrease normally during the incubation period.

Nembutal and Isoniazid. Although narcotics were shown[6,9] to inhibit the acetylation of sulfonamides by liver, the mechanism of the inhibition is controversial. Isoniazid was reported[10] to inhibit competitively the acetylation of sulfonilamide by liver. The effect of such compounds upon the acetylating activity in rat blood was studied; the results in Table 5 show that nembutal exhibited a marked inhibition of

TABLE IV
Effect of Fluoride on Acetylation by Normal Rat Blood

Experiment Number	Concentration of Sodium Fluoride (M)	Concentration of Glucose (M)	% Acetylation in 24 hours
1	0	0	14
2	0	0.1	31
3	1.8×10^{-2}	0	4
4	3.6×10^{-2}	0	3
5	3.6×10^{-2}	0.1	7

TABLE V
Effect of Nembutal on Acetylation by Rat Blood

Experiment Number	*Additions to Normal Blood*	*% Acetylation*
1, 2	None	15, 16
3, 4	Nembutal* (10^{-3} M)	7, 8
5, 6, 7	Nembutal (4×10^{-3} M)	2, 3, 2
8, 9	Nembutal (5×10^{-3} M)	0, 0
10, 11	Nembutal (5×10^{-3} M)+ ATP (6×10^{-3} M)	5, 3
12	Nembutal (10^{-3} M)+ CoASH (10^{-3} M)	7

*Final concentration in blood.

PABA acetylation. With a drug concentration of 5 x 10^{-3} M, the rat blood did not acetylate at all. Further, this inhibitory effect was not alleviated by the addition of ATP or CoASH.

Isoniazid however was found to have no appreciable influence at a concentration of 0.001 M upon PABA acetylation.

Cortisone Acetate and 9-Fluorocortisone. Recent reports[11] of the positive effects of certain adrenal cortex hormones upon the *in vitro* acetylation of sulfonilamide by liver prompted a study of the effect of adrenalectomy on the PABA acetylation by rat blood. Adult rats were adrenalectomized and maintained on salt water for a period of 5 five days. At the end of this period, blood of these rats was tested for acetylation. The results indicate that adrenalectomy had no significant influence on normal blood acetylation values. Likewise, the addition of anti-inflammatory agents such as cortisone and 9-fluorocortisone failed to show any effect.

DISCUSSION

The blood is a heterogeneous mixture of fluid plasma and cellular elements, erythrocytes, leukocytes and platelets. Erythrocytes and platelets are known to possess active glycolytic enzymes and coenzymes.[12,13] The presence of several of the glycolytic enzymes and most of the citric acid cycle enzymes recently has been demonstrated in leukocytes.[14] A mixture of all three of the cellular constituents, such as in whole blood, therefore may be capable of metabolizing glucose completely. This may lead to the production of energy, by way of ATP synthesis, provided there are respiratory chain mechanisms in blood. The evidence, however, for the existence of cytochromes and cytochrome oxidase in blood is lacking. Because the biological acetylation is known to be energy dependent, the present study indicates that probably energy is liberated during endogenous blood glucose metabolism and at least part of this energy may be utilized by the acetylating systems in blood.

A marked increase in acetylation followed the addition of glucose to rat blood. Addition of ATP however did not appreciably alter normal acetylation values. This might be due to a combination of factors such as the impermeability of cell membranes to ATP, low ATP concentrations in the reaction mixture and hydrolysis of ATP by the powerful ATPase of the erythrocyte stroma.

The fluoride inhibition of acetylation apparently is due to its blocking of enolase activity which would limit severely the concentration of available substrate as well as energy for the acetylation reactions. Blood glucose, in presence of the fluoride ion, probably is metabolized to phosphoglyceric acid; this would explain the di-

minished amount of reducing substance in the blood.

The disaccharides, sucrose, maltose and lactose, did not influence the acetylation values in human or rat blood. These observations are in agreement with the view that disaccharides are split to monosaccharides by the intestinal enzymes before being absorbed into the blood stream, and that the body has little ability to utilize disaccharides injected into the blood.

Adrenalectomy of rats had no effect on their blood acetylation values and these results are compatible with the observations of Dumm and Ralli[15] on the effect of severe adrenal insufficiency on the *in vivo* acetylation of PABA in rats.

On the basis of these results it is obvious that blood glucose and/or some of the intermediates of glycolysis are important factors in the acetylating activity of blood. Insofar as Ostrom[16] has shown that blood sugar levels are consistently higher in individuals suffering from periodontal disease, the higher acetylating activity is this type of blood may be entirely due to the glucose concentration. This phase of the problem requires further investigation.

SUMMARY

The influence of several chemical factors upon the acetylating activity of blood was studied. Glucose and fructose-6-phosphate at 0.1 M concentration increased the acetylation of PABA while fructose, galactose, sucrose, maltose and lactose did not alter appreciably the normal acetylating activity of rat blood. The addition of ATP, acetate and CoASH individually or in combination did not affect the normal acetylation by rat blood.

Fluoride at 0.018 M concentration inhibited 75% of acetylation, while nembutal at a concentration of 0.005 M completely inhibited the acetylation of PABA by rat blood.

Adrenalectomy of rats had no influence on their blood acetylation values.

REFERENCES

1. Hue, A., Burrill, D. and Fosdick, L. S.: A Correlation of Blood Constituents of Periodontal Disease. (1) Acetylating Activity of Blood.

2. Hensel, M.: Quantitative Studien Uber Acetylierungsprozesse Im Tierkorper, Z. physiol. Chem. 93:401-411, 1915.

3. Harrow, B., Power, F. W., and Sherwin, C. P.: Acetylation, Proc. Soc. Exper. Biol. Med. 24:422-424, 1927.

4. Klein, J. R., and Harris, J. S.: The Acetylation of Sulfanilamine in Vitro, J. Biol. Chem. 124:613-625, 1938.

5. Lipmann, F.: Acetylation of Sulfanilamide by Liver Homogenates and Extracts, J. Biol. Chem. 160:173-189, 1945.

6. Johnson, W. J., and Quastel, J. H.: Narcotics and Biological Acetylations, Nature 171:602-5, 1953.

7. Blondheim, S. H.: Acetylation by Blood Cells, Arch. Biochem. and Biophys. 55:365-72, 1955.

8. Folin, O., and Wu, H.: A System of Blood Analysis. I. A Simplified and Improved Method for Determination of Sugar, J. Biol. Chem. 41:367-374, 1920.

9. McLennan, H., and Elliott, K. A. C.: Factors Affecting Synthesis of Acetylcholine (ACh) by Rat Brain Cortex Slices, Federation Proc. 9:202-3, 1950.

10. Makino, K., Kinoshita, T., and Itoh, T.: Biological Acetylation of Iso-Nicotinyl Hydrazide, Nature 173-36, 1954.

11. Franz, J. M., and Lata, G. F.: Hormonal Effects upon Sulfanilamide Acetylation In Vitro and In Vivo, Federation Proc. 12:204, 1953.

12. Bartlett, G. R., and Marlow, A. A.: Erythrocyte Carbohydrate Metabolism. I. The Flow of C^{14}-Glucose Carbon into Lactic Acid, Carbon Dioxide, Cell Polymers and Carbohydrate Intermediate Pool, J. Lab. Clin. Med. 42:178-87, 1953.

13. Campbell, E. W., Small, W. J., and Dameshek, W.: Metabolic Activity of Human Blood Platelets, J. Lab. Clin. Med. 47:835-43, 1956.

14. Vercauteren, R.: A Study of Leucocyte Metabolism by the Tetrazolium Reduction Method, Enzymol. 18:97-110, 1957.

15. Dumm, M. E., and Ralli, E. P.: The Influence of Sex and Diet on the Capacity of Intact and Adrenalectomized Rats to Acetylate p-Aminobenzoic Acid, J. Nutrition 44:265-74, 1951.

16. Ostrom, C. A., Switzer, J. L., and Fosdick, L. S.: Chemical Studies in Periodontal Disease VII. Correlation of Blood Glucose Tolerance and Tissue Glycogen Concentration in Periodontal Disease, J. Am. Dent. Assoc. 42, 388, 1951.

Factors that Influence the Acetylating Activity of Blood

2. Effect of Vitamin Deficiency upon Acetylating of PABA

by D. Subrahmanyam, R. Quentin Blackwell and L. S. Fosdick, *Chicago, Illinois*

INSOFAR as it has been found that deficiencies of certain members of the Vitamin B Complex, Vitamin C and other vitamins may be partially responsible for periodontal disease, and insofar as blood acetylating activity appears to be associated with periodontal disease,[1] it was thought of interest to determine the acetylating activity of the blood from normal animals and from animals suffering from various dietary deficiencies.

Numerous workers have studied the effect of vitamin deficiencies on the acetylation of substrates in vivo.[2-8] This has been done by injecting PABA* or sulfanilamide into the vitamin deficient animal followed by the measurement of the amount of acetylated derivative excreted in the urine. The acetylation was attributed solely to the liver. As yet no one has studied the effect of dietary deficiencies on blood acetylating activity.

MATERIALS AND METHODS

Deficiencies in vitamins of the B complex were produced in adult male albino rats while adult female guinea pigs were used in the ascorbic acid deficient experiments. All of the vitamin test diets and vitamins used in these experiments were obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio. The animals were kept in individual cages and fed with vitamin deficient diets until they became severely deficient in the respective vitamins, as judged by the characteristic deficiency symptoms. Diets deficient in one of each of the following vitamins were fed to rats: thiamine, niacin, pantothenic acid, riboflavin, biotin, B_{12}, folic acid, and pyridoxine. The ascorbic acid deficient diet was fed to guinea pigs. The blood from vitamin deficient rats was drawn by cardiac puncture. The blood was tested for PABA acetylating activity by the method previously described.[1] Control experiments were carried out simultaneously on normal rat blood.

RESULTS

Thiamine Deficiency. The acetylating activity of thiamine deficient rat blood was compared with that of normal rat blood; both were studied with and without supplementation by cocarboxylase and by glucose. The results presented in Table 1 show that thiamine deficiency resulted in acetylation of about 12% normal value. Addition of cocarboxylase at a concentration of about 3×10^{-3} M restored the acetylation by thiamine deficient rat blood to 80% normal value. Cocarboxylase at this concentration was, however, found to have no effect on the acetylation by normal rat blood. Supplementing the avitaminosis rat blood with glucose at a final concentration of 0.08 M increased the acetylation to an average of 14%. The increase, however, was only one-half as much as the increase obtained in normal rat blood in the presence of glucose at the same concentration.

Niacin Deficiency. The data on the effect of niacin deficiency is given in Table 2. Deficiency in this vitamin led to an 80% loss of normal acetylating activity while the addition of DPN or TPN alone or in combination to the vitamin deficient rat blood restored the activity by 60%. However, DPN, at the concentration used, showed no influence on normal acetylat-

*The following abbreviations are used: PABA, p-aminobenzoic acid; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; Co-ASH, coenzyme A; and TPP, thiamine pyrophosphate.

This study was supported in part by grant No. D 395-C-1 from U. S. Public Health Service. This report is from a dissertation submitted to the faculty of the Graduate School of Northwestern University by D. Subrahmanyam in partial fulfilment of the requirements of the degree of Doctor of Philosophy.

TABLE I
Effect of Thiamine Deficiency upon Acetylation

Normal Rat Blood			*Thiamin Deficient Rat Blood*		
Experiment Number	*Addition to the Blood*	*Acetylation %*	*Experiment Number*	*Additions to the Blood*	*Acetylation %*
1,2,3,4,5	None	13,13,13, 14,13	10,11,12,13	None	0,2,4,0
6,7	TPP 2.7×10^{-3}M	12,15	14,15,16,17	TPP 2.7×10^{-3}M	11,11,9,11
8,9	Glucose 0.08M	28,27	18,19,20,21	Glucose 0.08M	15,14,17,17

ing figures of rat blood. The addition of glucose had no effect on the niacin deficient blood.

Pantothenic Acid Deficiency. Pantothenic acid deficient rat blood, as revealed by the results shown in Table 3, possessed only 40% normal capacity to acetylate PABA. Addition of CoASH or pantothenate to the vitamin deficient rat blood resulted in an increase of two to three times the normal value. Glucose, at 0.05 M concentration, improved the acetylation by vitamin deficient rat blood although not to the same extent as by blood from normal rat. It is interesting to note that addition of identical amounts of CoASH to normal rat blood did not appear to alter the normal acetylating activity.

Riboflavin Deficiency. The results on the acetylation by riboflavin deficient rat blood are given in Table 4. Deficiency of this vitamin in rat blood led to a loss of more than one-half of the normal acetylating ability. This loss was not found to improve on supplementing the vitamin deficient blood with riboflavin. Addition of glucose at 0.1 M concentration improved the acetylation by the riboflavin deficient rat blood considerably, although the increase was not as much as in the case of normal rat blood supplemented by the same amount of sugar.

Biotin Deficiency. Biotin deficiency, as reported in Table 5, resulted in a cessation of blood acetylating activity. This total inhibition was not altered by the *in vitro* addition of biotin (4×10^{-3} M) or glucose (0.1 M). Biotin at this concentration appeared to have no effect on the extent of acetylation by normal rat blood.

TABLE II
Effect of Niacin Deficiency upon Acetylation

Experiment Number	*Normal Rat Blood*		*Niacin Deficient Rat Blood*		
	Additions to the Blood	*Acetylation (%)*	*Experiment Number*	*Additions to the Blood*	*Acetylation (%)*
1,2	None	17,15	5,6,7,8,9,	None	1,1,3,3,5
3,4	DPN(2.4×10^{-3}M)	13,17	10	DPN(6×10^{-4}M)	10
			11	DPN(1.2×10^{-3}M)	11
			12	DPN(2.4×10^{-3}M)	10
			13	DPN(1.9×10^{-3}M) + TPN(1.7×10^{-3}M)	12
			14,15	TPN(1.3×10^{-3}M)	10,9

TABLE III
Effect of Pantothenic Acid Deficiency upon Acetylation

Normal Rat Blood			*Pantothenic Acid Deficient Rat Blood*		
Experiment Number	*Additions to the Blood*	*Acetylation (%)*	*Experiment Number*	*Additions to the Blood*	*Acetylation (%)*
1,2	None	15,15	6,7,8,	None	8,5,5
3	Glucose(0.05M)	25	9,10	Glucose(0.05M)	19,20
4,5	CoASH (1.4×10^{-4}M)	15,14	11,12	CoASH (1.2×10^{-4}M)	33,37
			13,14	CoASH (2.3×10^{-4}M)	45,49
			15	CoASH (3.4×10^{-4}M)	48
			16	Pantothenate (3.6×10^{-3}M)	32
			17	Pantothenate (7.1×10^{-3}M)	30

Folic Acid and B_{12} Deficiencies. Blood samples from vitamin B_{12} deficient rats and folic acid deficient rats were tested for their acetylating activities. It was found that normal blood acetylation values were not changed appreciably by deficiencies in either of the vitamins.

Pyridoxine Deficiency. The blood from the pyridoxine deficient animals acetylated about 40% normal value (Table 6). Glucose (0.1 M) stimulated the acetylating activity of the avitaminosis rat blood as in the case of normal rat blood.

Ascorbic Acid Deficiency. The results of the acetylation studies on the blood from the ascorbutic guinea pigs are presented in Table 7. These results indicate that ascorbic acid deficiency decreases the extent of acetylation by guinea pig blood to about ⅓ of its normal value. Addition of vitamin C to the deficient guinea pig blood did not raise the value to a significant extent.

DISCUSSION

The presence of several of the glycolytic enzymes and most of the tricarboxylic acid cycle enzymes has been demonstrated recently in blood cells.[9, 10, 11] It is known that many of these enzymes are dependent upon the presence of coenzymes for their activity and that some of the B complex vitamins form integral parts of certain co-

TABLE IV
Effect of Riboflavin Deficiency upon Acetylation

Normal Rat Blood			*Riboflavin Deficient Rat Blood*		
Experiment Number	*Additions to the Blood*	*Acetylation (%)*	*Experiment Number*	*Additions to the Blood*	*Acetylation (%)*
1,2,3,4	None	14,20,19,16	9,10,11,12	None	6,6,7,5
5,6	Riboflavin (2.7×10^{-3}M)	18,18	13,14,15	Riboflavin (2.7×10^{-3}M)	8,8,8
7,8	Glucose(0.1M)	28,32	16,17	Glucose(0.1M)	17,14

TABLE V
Effect of Biotin Deficiency upon Acetylation

Normal Rat Blood			*Biotin Deficient Rat Blood*		
Experiment Number	*Additions to the Blood*	*Acetylation (%)*	*Experiment Number*	*Additions to the Blood*	*Acetylation (%)*
1,2	None	13,12	5,6	None	0,0
3,4	Biotin(4×10^{-3}M)	12,12	7,8	Biotin(4×10^{-3}M)	0,0
			9,10,11	Glucose(0.1M)	6,0,0

enzymes. Deficiency in these vitamins would lead, consequently, to a reduction in the coenzyme levels of the blood, thereby affecting glucose metabolism in the tissues, including the blood, and consequently an important series of energy producing reactions would be disturbed. The data presented here shows that acetylation of PABA by blood was inhibited when the rats were deficient in the various vitamins.

The results on the pantothenic acid deficient rat blood indicate that the activity of the "acetylase" may be higher in this blood than in normal rat blood. This would, perhaps, explain the increase of 2 to 3 times normal acetylating values by the vitamin deficient rat blood when supplemented with CoASH or pantothenate.

This may also explain the high acetylating activity in the blood from periodontal cases. Even if such cases are partially deficient in pantothenic acid, high blood sugars will definitely elevate the acetylating activity.

The precise role of biotin in the metabolism is not well established. Recently, Lichstein and Boyd[12] indicated that biotin is required in the assimilation of glucose by saccharomyods cerevisiae. Williams et al.[13] confirmed these results and proposed that either biotin functions as a coenzyme in the phosphorylation of glucose (or other substrates acceptable to hexokinase) or, alternately, it functions, directly or indirectly in the cellular synthesis of hexokinase. The results obtained in the present experiments lend support to the view that biotin is concerned with carbohydrate metabolism but give no indication as to the actual mechanism.

The mechanism of the effect of pyridoxine on acetylation is not quite clear. However, in recent years there has been increasing evidence[14,15] that pyridoxine is involved in the biosynthesis of CoASH; specifically, it is believed to be involved in the decarboxylation of pantothenyl cysteine to pantotheine.

It appears from these results that the acetylation of PABA by rat blood was affected by certain compounds that influence the formation of acetyl groups ATP or

TABLE VI
Effect of Pyridoxine Deficiency upon Acetylation

Normal Rat Blood			*Pyridoxine Deficient Rat Blood*		
Experiment Number	*Additions to the Blood*	*Acetylation (%)*	*Experiment Number*	*Additions to the Blood*	*Acetylation (%)*
1,2,3	None	15,17,15	4,5,6,7,8	None	5,4,9
				Glucose(0.1M)	28,25

TABLE VII
Effect of Ascorbic Acid Deficiency upon Acetylation

Normal Guinea Pig Blood			*Ascorbic Acid Deficient Guinea Pig Blood*		
Experiment Number	*Additions to the Blood*	*Acetylation (%)*	*Experiment Number*	*Additions to the Blood*	*Acetylation (%)*
1,2,3,4	None	20,18,16,17	5,6,7,8, 9,10,11	None Vitamin C(0.05M)	8,4,10,8 9,12,10

CoASH. It appears likely that certain metabolic disorders arising from some of these vitamin deficiencies may be due in part at least, to their affect on acetylation. Their relation to periodontal disease may also be through some such mechanism.

SUMMARY

The blood acetylating activity of animals deficient in thiamine, niacin, pantothenic acid, riboflavin, biotin, B_{12}, folic acid, pyridoxine and ascorbic acid was measured and compared to that in normal animals. It was found that deficiencies of all of the above with the exception of folic acid and B_{12} caused a marked decrease in blood acetylating activity. In most cases the in vitro addition of the vitamin under test resulted in restoring the activity. In the case of pantothenic acid deficient rats and in vitro restoration of the vitamin or the addition of CoASH resulted in an acetylating activity about double the normal value.

BIBLIOGRAPHY

1. Hue, A. C., Burrill, D. Y. and Fosdick, L. S., A Correlation of Blood Constituents with Periodontal Disease, Chemistry Department, Northwestern University Dental School, Chicago, Illinois.

2. Jung, A., Die Funktionen Der Vitamins Des B-Komplexes Im Organismus, Z. Vitaminf. Beihefte No. 1:1-114, 1941.

3. Lipmann, F., Acetylation of Sulfanilamide by Liver Homogenates and Extracts, J. Biol. Chem. 160:173-189, 1945.

4. Biggs, T. R., and Hegsted, D. H., The Effect of Pantothenic Acid Deficiency and Acetylation in Rats, J. Biol. Chem. 172:539-45, 1948.

5. Biggs, T. R., and Hegsted, D. H., Some Factors Affecting the Acetylation of p-Aminobenzoic Acid in the Rat, J. Biol. Chem. 178:669-75, 1949.

6. Fatterpaker, P., Marfatia, U., and Sreenivasan, A., Protective Action of Vitamin B_{12} in the Hyperthyroid Rat, Nature 176:165, 1955.

7. Belavady, B., and Banerjee, S., Metabolism of Cholesterol in Scorbutic Guinea Pigs, J. Biol. Chem. 209:641-5, 1954.

8. Blondheim, S. H., Acetylation by Blood Cells, Arch. Biochem. and Biophys. 55:365-72, 1955.

9. Bartlett, G. R., Savage, E., Hughes, L., and Marlow, A. A., Carbohydrate Intermediates and Related Cofactors in the Human Erythrocute, J. Applied Physiol. 6:51-6, 1953.

10. Campbell, E. W., Small, W. J., LoPilato, E., and Dameshek, W., Glycolytic Intermediates of Human Platelets: Their Separation and Identification, Proc. Soc. Exptl. Biol. Med. 94:505, 1957.

11. Vercauteren, R., A Study of Leucocyte Metabolism by the Tetrazolium Reduction Method, Enzymol. 18:97-110, 1957.

12. Lichstein, H. C., and Boyd, R. B., The Dual Role of Biotin in Glucose Metabolism of Saccharomyces Cerevisine, Arch. Biochem. and Biophys., 55:307-9, 1955.

13. Williams, V. R., Andrews, E. A., and Christman, J. F., Studies on a Biotin-Hexokinase Interrelationship, Arc. Biochem. and Biophys., 66: 234-39, 1957.

14. Terroine, T., and Andrian, J., Les Interrelations Vitaminiques Carence En Pyridoxine et Teneurs Des Tissue En Riboflavine, Nicaine et Acids Pantothenique, Arch. Sci. Physiol., 4:435-44, 1951.

15. Yamada, K., Sawaki, S., and Hayami, S.: Participation of Vitamin B_6 in the Biosynthesis of Coenzyme A., J. Vitaminol., 2:296-8, 1956.

Gingival Response to the Systemic Administration of Sodium Diphenyl Hydantoin (Dilantin) in Cats*

by JUN ISHIKAWA,** AND IRVING GLICKMAN, *Boston, Mass.*

HYPERPLASIA of the gingiva was reported as a side effect of the use of Dilantin (Sodium diphenylhydantoin) shortly after the drug was introduced for the treatment of epilepsy.[1,2] The clinical, histopathological and histochemical features of the gingival changes in Dilantin-treated individuals have been described by numerous investigators.[3-8] However from observations in humans after the lesions have developed, it has been difficult to determine the manner in which the gingival hyperplasia is initiated and the extent to which local irritation is a causative factor. Numerous animal experiments[8-23] have been conducted in an attempt to clarify these and other aspects of the gingival changes, but many questions have remained unanswered. We were particularly interested in the histological changes involved in the initiation and enlargement of the gingiva, the region of the periodontium in which the lesion originated and its relationship to local irritation.

PROCEDURE

Nineteen mongrel cats, 16 females and 3 males with permanent dentition, ranging in weight from 1700 to 4000 grams were used. The animals were arranged in two groups. In Group I, there were 5 cats each of which served as both a control and experimental animal. A biopsy specimen of the maxillary left second or third incisor consisting of a tooth with bone and gingiva in situ was taken in each animal before Dilantin was administered. Similar specimens of other areas were taken at intervals of 5, 7, 18, 25 and 28 days after administration of drug was begun. This provided a method for studying the initiation and progressive development of the gingival changes in individual animals.

In Group II (fourteen animals), eleven received Dilantin and three served as controls. Dilantin was administered from 5 to 166 days. Specimens of teeth with bone and gingiva in situ were obtained at time of sacrifice, or at autopsy in the case of 6 animals which died during the experiment.

All animals were fed a diet of Purina Chow in a mixture of softened and hard pellets so as to provide functional stimulation to the gingiva. Dilantin was fed orally in capsule in a daily dosage of 7 mgs/kilo of body weight.

Observations were made of the gingiva at the outset of the experiment and each day thereafter. In addition to tooth and attached periodontium, specimens of buccal mucosa and vagina were studied. The tissues were prepared in serial section. The formic acid—sodium citrate method was used for decalcification.

GROSS OBSERVATIONS

In all animals, experimental and controls, there was some erythema of the gingival margin and interdental papillae at the outset of the experiment. This was most apparent in the maxillary canine areas and occurred to lesser degree in the mandibular canine, and maxillary and mandibular incisor areas.

Following administration of Dilantin the initial gross gingival changes were marginal and interproximal erythema, ac-

*From the Ziskin Memorial Research Laboratory, Department of Periodontology, Tufts University School of Dental Medicine, Boston, Mass.

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**Present address, Tokyo Medical and Dental University, Tokyo, Japan.

centuation of pre-existent erythema and enlargement of the gingival margin. The marginal capillaries were dilated and formed a brush-like pattern at the margin (Fig. 1). Erythematous enlargement of the gingiva was followed by the extrusion of small vascular bead-like masses, interproximally, labially and lingually. These masses appeared to protrude from the space between the gingiva and the teeth and extended onto the tooth surface. They were attached to the gingival margin but demarcated from it by a thin linear depression.

The marginal gingiva along with the bead-like projections increased in size and progressively diminished in redness. The ultimate result was pronounced enlargement of the gingiva interproximally, labially and lingually which encircled the individual teeth and covered varying portions of the crown (Fig. 2). The enlarged gingiva was essentially normal in color, firm and with a minutely lobulated surface.

The gingival changes described above occurred in 8 of the 16 Dilantin-fed animals. The initial changes became apparent at different intervals after the administration of Dilantin was begun, as follows: 5 days (2 animals); 6 days (3 animals); 7 days (one animal); 13 days (one animal) and 21 days (one animal). The maxillary third incisor and canine areas were most often the sites of initial involvement. Ultimately the gingiva in relation to all the teeth became enlarged. However, the enlargement was most pronounced in the aforementioned areas. The rate of enlargement varied from animal to animal and in different areas in the same animal.

When a tooth with enlarged gingiva was removed for biopsy purpose, the resultant edentulous area presented no abnormal mucosa changes.

In the Dilantin-fed animals, in which gingival enlargement did not occur, and in the control animals, there were some changes in the degree of gingival erythema in the course of the experimental period.

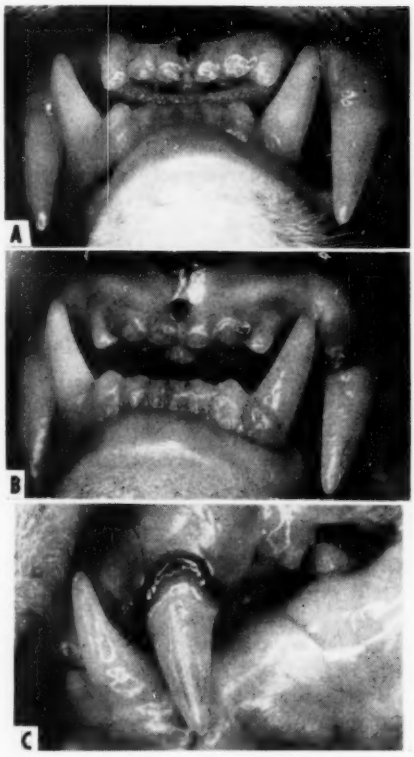


Fig. 1. Experimental animal #16. A, before administration of Dilantin. B, 15 days after administration of Dilantin. There is marginal and interproximal enlargement of the gingiva in maxillary incisor and canine areas. Note the brush-like pattern of prominent capillaries. C, 15 days after administration of Dilantin. Lateral view showing erythematous marginal enlargement.

MICROSCOPIC OBSERVATIONS

In the Dilantin-fed animals in which gingival enlargement did not occur and in the control animals the marginal gingiva presented varying degrees of leukocytic infiltration and edema with proliferation and degeneration of the epithelium of the marginal gingiva (Fig. 3).

In animals of Group I biopsies were obtained of teeth with attached periodontium before and during the administration of Dilantin. In this group it was possible to study the early stage of the gingival enlargement and trace its progress during the

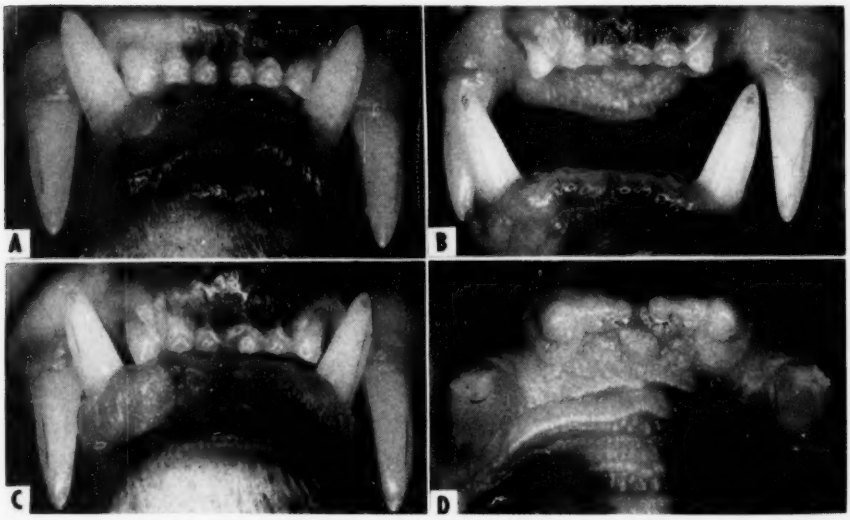


Fig. 2. Experimental animal #10. A, before administration of Dilantin. There is marginal erythema in maxillary canine areas. B, 62 days after administration of Dilantin, showing gingival enlargement in maxilla and mandible. Note minutely lobulated surface of the enlarged gingiva. C, 166 days after administration of Dilantin, showing pronounced marginal enlargement. The gingiva is firm, with less edema and erythema than in B. D, 166 days after administration of Dilantin. Palatal view showing pronounced enlargement with minutely lobulated surface.

experimental period. In Group II, material for microscopic study was obtained from Dilantin-fed and control animals at the termination of the experimental period.

In the Group I animals, before Dilantin was administered, a slight to moderate infiltration of leukocytes was present in the connective tissue at the base of the gingival sulcus, without any other notable changes (Figs. 4 and 5).

There was considerable variation in the microscopic features of the gingival enlargement examined at comparable intervals after Dilantin administration was begun. However, the following sequence of microscopic changes was discernible in the initiation and progressive development of the gingival lesions.

INITIAL STAGE

The earliest changes occurred in the connective tissue core of the gingival margin and interproximally, and consisted of proliferation of fibroblasts and the formation of numerous dilated and engorged capillaries interspersed among thick strands of collagen (Figs. 6 and 7). The location of the initial connective tissue activity was unrelated to the inflammation at the base of the sulcus, and in several instances occurred in animals with only slight leukocytic infiltration. The central connective tissue mass increased in size, expanded the gingiva and compressed the epithelium lining the gingival sulcus, and the crest of the gingival margin (Figs. 8 and 9). The epithelial cells in the latter areas were separated and fragmented.

SECOND STAGE

The mass of proliferating fibroblasts, collagen and capillaries expanding along the tooth surface and wedging into the adjacent gingiva, occupied the resultant space between the tooth and the gingiva and projected beyond the crest of the gingival margin into the oral cavity (Figs. 10 and 11). There were remnants of degenerated epithelial cells along the inner

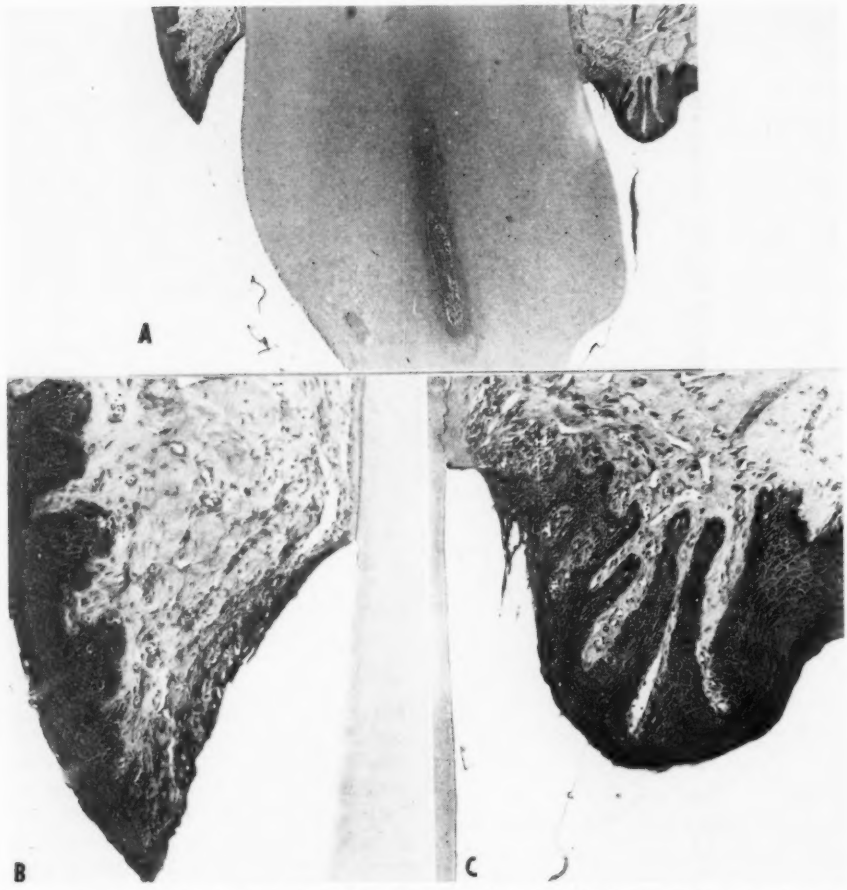


Fig. 3. Experimental animal #5. A, 62 days after administration of Dilantin. Labio-palatal section through the maxillary right third incisor. There is slight inflammatory involvement but no enlargement. (Compare with Fig. 4. Same animal, before Dilantin was administered). (Hematoxylin and eosin; orig. mag. 60X). B, detailed view of the labial gingival margin shown in Fig. 3-A. Note dilated capillaries. C, detailed view of the palatal gingival margin shown in Fig. 3-A. Note slight leukocytic infiltration. (Hematoxylin and eosin: orig. mag. 170X).

surface of the connective tissue mass adjacent to the tooth and at the crest which projected into the oral cavity. Isolated leukocytes were scattered throughout the extruded connective tissue mass. The gingiva adjacent to, and wedged aside by the protruding connective tissue mass was clearly demarcated from it. The gingival epithelium was hyperplastic and extended along the lateral border of the protruding mass.

FINAL STAGE

This stage was characterized by the formation of a bulbous marginal and interproximal enlargement of the gingiva (Fig. 12). Inflammation of the sulcus was more pronounced than in the earlier stages and was concentrated at the base of the deepened sulcus formed by the enlarged gingiva. Leukocytes were interspersed in dense accumulations of fibroblasts and capillaries in the inflamed areas (Fig. 13).

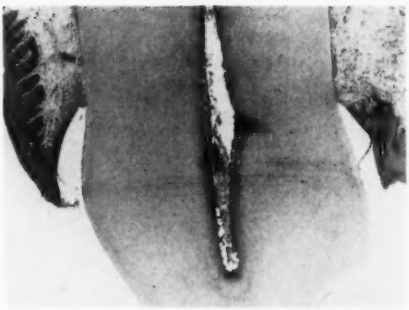


Fig. 4. Experimental animal #5, before administration of Dilantin, labio-palatal section through the maxillary left third incisor. (Hematoxylin and eosin: orig. mag. 70X).

In the remaining connective tissue which constituted the major portion of the enlarged gingiva, inflammatory involvement was slight. The appearance of the connective tissue differed from specimen to specimen and in different areas of the same specimen. It varied from that of a mixture of young fibroblasts and capillaries in a meshwork of collagen strands (Fig. 14) to that of a dense relatively acellular mass of well formed collagen bundles with few capillaries (Figs. 15 and 16).

The epithelium covering the enlarged mass was well formed, continuous with the adjacent gingiva and hyperplastic. The epithelium lining the sulcus presented varying degrees of edema and leukocytic infiltration. In some specimens there was exudate in the gingival sulcus.

No significant microscopic alterations were observed in the periodontal membrane, buccal mucosa or vaginal mucosa of the Dilantin-fed animals.

DISCUSSION

Gingival enlargement occurred in 50% of the Dilantin-fed animals. Based upon the microscopic findings it was possible to divide the enlargement into an initial, second and final stage.

Fig. 5. Experimental animal #5, before administration of Dilantin, detailed view of labial gingival margin shown in Fig. 4. Note slight leukocytic infiltration in connective tissue at base of sulcus. (Hematoxylin and eosin: orig. mag. 200X).

Fig. 6. Experimental animal #12, 18 days after administration of Dilantin. Labial marginal gingiva of the maxillary right third incisor in initial stage of enlargement. There is only slight leukocytic infiltration at the base of the sulcus. (Hematoxylin and eosin: orig. mag. 200X).

Fig. 7. Experimental animal #12, 18 days after administration of Dilantin, detailed view of labial gingival margin shown in Fig. 6. Note the proliferation of fibroblasts and the formation of numerous dilated and engorged capillaries interspersed among thick strands of collagen. The connective tissue has enlarged to the crest of the margin. The overlying epithelium is absent. (Hematoxylin and eosin: orig. mag. 400X).

Fig. 8. Experimental animal #16, same animal as shown in Fig. 1, 20 days after administration of Dilantin. Labio-palatal section of palatal marginal gingiva of the maxillary right third incisor. The central connective tissue mass increased in size, expanded the gingiva and compressed the epithelium lining the gingival sulcus. (Hematoxylin and eosin: orig. mag. 220X).

Fig. 9. Experimental animal #16, 20 days after administration of Dilantin. Detailed view of palatal gingival margin shown in Fig. 8. Note the fibroblastic activity with formation of dilated and engorged capillaries. (Hematoxylin and eosin: orig. mag. 800X).

Fig. 10. Experimental animal #13, 5 days after the administration of Dilantin. Labio-palatal section of palatal marginal gingiva of the maxillary right third incisor. The gingiva is wedged aside and the connective tissue has protruded beyond the margin. (Hematoxylin and eosin: orig. mag. 250X).

Fig. 11. Experimental animal #13, 5 days after the administration of Dilantin. Detailed view of palatal gingival margin shown in Fig. 10. Note the proliferating fibroblasts, collagen and capillaries. Remnants of degenerated epithelial cells are seen along the inner surface of the connective tissue adjacent to the tooth surface. The gingiva adjacent to this extruding mass is clearly demarcated from it and acanthotic. (Hematoxylin and eosin: orig. mag. 400X).

Fig. 12. Experimental animal #10 (same animal as shown in Fig. 2D), 166 days after administration of Dilantin. Labio-palatal section of the maxillary right third incisor with bulbous marginal enlargement of the gingiva. Leukocytic infiltration is concentrated at the base of the sulcus. Note hyperplastic epithelium. (Hematoxylin and eosin: orig. mag. 70X).

The initial stage appears in the connective tissue of the gingiva as a localized dense accumulation of proliferating fibroblasts and numerous capillaries in a meshwork of collagen strands. In the second stage it is an expanded vascular connective tissue mass, wedged between the tooth surface and adjacent gingiva and protruding beneath the margin of the gingiva into the oral cavity. In the final stage it appears as a discrete bulbous marginal and interproximal densely collagenous connective tissue mass covered by hyperplastic stratified squamous epithelium.

The gross appearance of the gingiva reflects the microscopic changes in the various stages. Initially the interproximal and marginal gingiva are slightly enlarged with engorged prominent capillaries. In the second stage, red bead-like masses appear as protrusions from under the gingival margin. A slight linear depression demar-

Fig. 13. Experimental animal #10, 166 days after administration of Dilantin. Detailed view of enlarged gingiva shown in Fig. 12, area near base of sulcus. Note leukocytes interspersed in dense accumulation of fibroblasts and capillaries. There is also inflammatory exudate in the sulcus. (Hematoxylin and eosin: orig. mag. 400X).

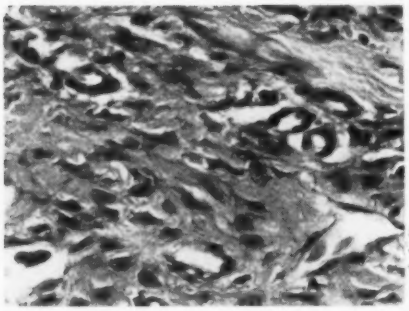


Fig. 14. Experimental animal #10, 166 days after administration of Dilantin. Detailed view of enlarged gingiva shown in Fig. 12. Area away from base of sulcus consists of a mixture of young fibroblasts and capillaries in a meshwork of collagen strands. (Hematoxylin and eosin; orig. mag. 80X).

Fig. 15. Experimental animal #10 (same animal as shown in Fig. 2C), 166 days after administration of Dilantin. Labio-palatal section of labial gingiva of the maxillary right third incisor, showing the bulbous marginal enlargement. Inflammation is concentrated near the base of the deepened sulcus formed by the enlarged gingiva. (Hematoxylin and eosin; orig. mag. 70X).

cates the protruding masses from the gingival margin. In the final stage, the enlarged gingiva extends labially, lingually and interproximally partially obscuring the teeth from view. When uncomplicated by inflammation the enlarged gingiva is pink and firm. The color and consistency change in the presence of accumulated local irritants.

The gingival enlargement associated with Dilantin is a marginal proliferative lesion. It affects only the marginal and interproximal gingiva, without appreciable extension into the adjacent attached gingiva or the underlying periodontal tissues.

Varying degrees of epithelial hyperplasia characterize the final stage of the gingival enlargement. One can only hypothesize regarding the extent to which the epithelial hyperplasia results from stimulation by the Dilantin or occurs in response to the connective tissue changes. As noted above, the changes which initiate the gingival enlargement occur in the connective tissue. Expansion of the connective tissue results in compression and degeneration of the epithelium at the crest of the gingiva and adjacent to the tooth surface. Only a few degenerated remnants of epithelial cells are present on the surface of the expanded connective tissue mass as it protrudes into the oral cavity. Proliferation of the epithelium of the adjacent gingiva is required to reconstruct the epithelial surface of the enlarged connective tissue mass.

We were interested in the manner in which inflammation was involved in the causation of the enlargement associated with Dilantin. This presents a problem because in all the animals examined microscopically before Dilantin was administered there was some evidence of inflammation even in areas with no gross inflammatory changes. It is not possible to determine whether inflammation is actually necessary for the initiation of the enlargement or whether it is simply an incidental finding.

Enlargement of the gingiva did not occur in eight of the Dilantin-fed animals even in the presence of inflammation. It would appear that regardless of the role of inflammation in initiating the enlargement associated with Dilantin, there are other factors which determine whether the lesion will occur.

Our findings do indicate that inflamma-

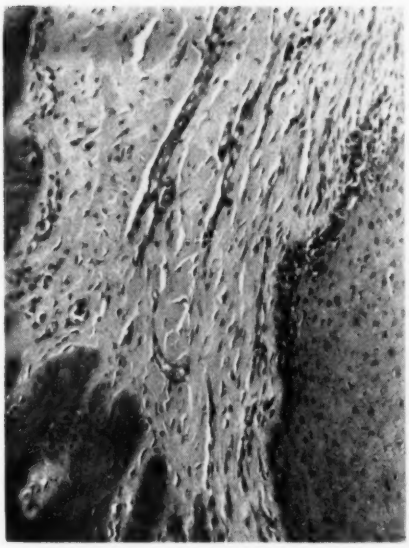


Fig. 16. Experimental animal #10, 166 days after administration of Dilantin. Detailed view of labial gingival margin shown in Fig. 15. The area near the margin consists of dense relatively acellular mass of well formed collagen bundles with few capillaries. (Hematoxylin and eosin; orig. mag. 400X).

tion alone does not initiate the fibroplastic gingival lesions. Gingival inflammation was present in some degree throughout the mouth of all the control animals. Gingival changes comparable to these observed in the Dilantin-fed animals did not occur.

We are led to the interpretation that inflammation is a complication of gingival enlargement associated with Dilantin rather than an initiating factor. In the initial stage, the connective tissue changes occur in an area of the gingiva considerably removed from the base of the sulcus where the inflammation is concentrated. Enlargement began in some Dilantin-fed animals with only the slightest microscopic evidence of inflammation but did not occur in others with pronounced grossly visible gingival inflammation. In the final stage of the enlargement local irritants in the deepened gingival sulcus lead to secondary inflammatory involvement. Pronounced microscopic inflammatory changes occur in relation to the base and inner surface of the sulcus with some spread of leukocytes and edema into the adjacent tissue. However, the major portion of the enlarged gingiva consists of a densely collagenous connective tissue stroma.

The mechanism whereby gingival enlargement is produced by Dilantin is not understood. The problem has been discussed extensively by Abadom.[24] Suggested as possible explanations for the gingival phenomenon are irritation to the gingiva by Dilantin[25] or a metabolized product[26] excreted in the saliva, irritation by Dilantin contained in blood plasma and red blood cells released by hemorrhage into the gingiva[27] and allergic response.[28-31] There is some disagreement regarding the latter concept.[32, 33] The peculiar susceptibility of the marginal and interproximal gingiva, the absence of changes in the remainder of the oral mucous membrane, the disappearance of the lesion after the teeth are extracted and the immunity of some individuals to Dilantin-induced gingival enlargement persist as provocative aspects of the problem.

CONCLUSIONS

Enlargement of the gingiva occurred in eight of sixteen cats fed Dilantin in a daily dosage of 7 milligrams per kilogram of body weight. The enlargement originates in the connective tissue of the marginal and interproximal gingiva as a proliferation of fibroblasts and capillaries in a meshwork of collagen strands. It is suggested that inflammation is a complicating rather than initiating factor.

The changes induced by Dilantin are confined to the interproximal and marginal gingiva. The attached gingiva, underlying periodontal tissues and remainder of the oral mucosa are not affected. No gross or microscopic changes were observed in edentulous areas created by the extraction of teeth in Dilantin-fed animals with gingival enlargement.

REFERENCES

1. Merritt, H. H. and Putnam, T. J.: Sodium Diphenyl Hydantoinate in the Treatment of Convulsive Disorders. J.A.M.A., 111:1068, 1938.

2. Kimball, O. P.: The Treatment of Epilepsy with Sodium Diphenyl Hydantoinate. J.A.M.A., 112:1244, 1939.

3. Esterberg, H. L. and White, P. H.: Sodium Dilantin Gingival Hyperplasia. J.A.D.A., 32:16, 1945.

4. Glickman, I. and Lewitus, M. P.: Hyperplasia of the Gingiva Associated with Dilantin (Sodium diphenyl hydantoinate) Therapy. J.A.D.A., 28:199, 1941.

5. Ishikawa, J.: Studies of Gingival Hyperplasia Associated with Sodium Diphenyl-hydantoin Therapy. (1) Clinical and Statistical Study. Japanese J. Conservative Dentistry (Nihon Hozon Shikagaku Zasshi), 2:147, 1959.

6. Stern, L., Eisenbud, L. and Klatell, J.: Analysis of Oral Reactions to Dilantin Sodium. J.D.Res., 22:157, 1943.

7. Staple, P. H.: Some Tissue Reactions Associated with 5:5-Diphenylhydantoin (Dilantin) Sodium Therapy. Brit. Dent. J., 95:289, 1953.

8. Ziskin, D. E., Stowe, L. R. and Zegarelli, E. V.: Dilantin Hyperplastic Gingivitis, 1) Its Cause and Treatment, 2) Differential Appraisal. Am. J. Orthodont. and Oral Surg. (Oral Surg. Sect.), 27:350, 1941.

9. Abadom, P. N.: The Pharmacological Reactions and Metabolism of 5:5-Diphenylhydantoin in the Ferret. (Thesis for M.S.), Univ. of Rochester, New York, 1959.

10. Bergman, A. G.: Parodontala Förhallanden hos vita Rattor, behandlade med Difenyl-hydantoin-Natrium, Svensk Tandläkare Tidskrift, 43:493, 1950.

11. Ennever, J. J. and Pendo, G. P.: The Effect of Sodium Diphenyl Hydantoinate (Dilantin) on the Gingivae of Swiss Mice. Washington Univ. Dent. J., 12:70, 1946.

12. Forscher, B. K. and Cecil, H. C.: Biochemical Studies on Acute Inflammation. II. The Effect of Dilantin. J.D.Res., 36:927, 1957.

13. Francis, L. E. and Melville, K. I.: Effect of Diphenylhydantoin (Dilantin) on Histamine Changes in the Gingival Tissue. J.Canad.Dent. Assn., 24:142, 1958.

14. Kajiya, K.: Experimental Study of Dilantin Gingival Hyperplasia. I. Experimental Observation of Continuous Dilantin Administration. II. The Effect of Continuous Dilantin Administration on Animal Growth. J. Kyushu Dent. Assn. (Kyushu Shika Gakkai Zasshi), 7 and 8: 1953 and 1954.

15. King, J. D.: Experimental Production of Gingival Hyperplasia in Ferrets Given Epanutin (Sodium Diphenylhydantoinate). Brit. J. Experim. Path., 33:491, 1952.

16. Ishikawa, G. and Shimada, Y.: Patho-histological Study on Gingival Hypertrophy due to Sodium Diphenyl Hydantoin Therapy. J.Japanese Stomatolog. Soc. (Kokubyo Gakkai Zasshi), 19:177, 1952.

17. Ishikawa, J.: Studies of Gingival Hyperplasia Associated with Sodium Diphenyl Hydantoin Therapy. II. Experimental Study of Dilantin Gingival Hyperplasia in Cats. Japanese J. Conservative Dent. (Nihon Hozon Shikagaku Zasshi), 2:169, 1959.

18. Maeda, E., Takenaka, K. and Fujii, T.: A Case Report of Dilantin Gingival Hyperplasia and Its Animal Experiment in Dogs. J. Hiroshima Dent. Assn., 1:3, 1954.

19. Schafer, W. C.: An attempt to Produce Gingival Hyperplasia in the Albino Rat by the Administration of Dilantin Sodium. J.D.Res., 27:768, 1948.

20. Schafer, W. G., Beatty, R. E. and Davis, W. B.: Effect of Dilantin Sodium on Tensil Strength of Healing Wounds. Proc. Soc. Exper. Biol., Med., N.Y. 98:348, 1958.

21. Staple, P. H.: Diphenyldantoin, Adrenal Function and Epilepsy. J.Endocrinol., 9:XVIII, 1953.

22. Staple, P. H.: Alkaline Phosphatase Activity in Gingiva of the Ferret. Brit. Dent. J., 102:305, 1957.

23. Swenson, H. M.: A Failure to Produce Enlargement of Gingiva in Rats by Using Dilantin Sodium. J.D.Res., 33:468, 1954.

24. Op. Cit. 9

25. Noach, E. L., Woodbury, D. M. and Goodman, L. S.: Studies on the Absorption, Distribution, Fate and Excretion of 4-C^{14}-Labelled Diphenylhydantoin. J.Pharmacol. and Exper. Therap., 122:301, 1958.

26. Brandon, S. A.: Treatment of Hypertrophy of the Gingival Tissue Caused by Dilantin Sodium Therapy. J.A.D.A., 37:732, 1948.

27. Strean, L. R. and Leoni, E.: Dilantin Gingival Hyperplasia—Newer Concepts Related to Etiology and Treatment. N.Y. State Dent. J. 25:339, 1959.

28. Op. Cit. 13

29. Van der Kwast, A. M.: Speculations Regarding the Nature of Gingival Hyperplasia due to Diphenylhydantoin Sodium. Acta Med. Scandinav., 153:399, 1956.

30. Gaillard, R. A.: Antihistamine Therapy for Gingival Hyperplasia due to Dilantin. New England J. Med., 256:76, 1957.

31. Melville, K. I.: The Dentist and the Endocrine Problem—Some Pharmacological Aspects. J.Dent.Med., 14:219, 1959.

32. Holowach, J., Thurston, D. L. and Gilster, J. E.: Antihistamine Therapy for Gingival Hyperplasia due to Diphenylhydantoin. New England J. Med., 259:180, 1958.

33. Ingle, J. I., Howard, W. L., Wash, K. and Zwick, H. H.: Effects of Antihistaminic Therapy on Diphenyl Hydantoin Sodium (Dilantin) Gingival Hyperplasia. J.A.D.A., 58:65, 1959.

Periodontal Manifestations of Cyclic Neutropenia

by D. Walter Cohen, D.D.S.* and Alvin L. Morris, D.D.S., PH.D.,** *Philadelphia, Pa.*

NEUTROPENIA, or agranulocytosis, is a condition characterized by a marked decrease or lack of circulating neutrophils in the peripheral blood. In some instances it develops without apparent cause, is resistant to treatment and is referred to as malignant or idiopathic neutropenia. It is now well established that a host of drugs can produce a reversible neutropenia due to their suppression of bone marrow activity. A rare variant of the disease, usually called cyclic neutropenia, is characterized by the periodic disappearance of circulating neutrophils. In its most typical form, the neutrophils completely disappear from or are markedly reduced in the peripheral blood approximately every three weeks.[29] After a five to eight day period, their numbers begin to increase but usually never represent more than 50 per cent of the differential white blood cell count.

In 1949 Reimann and DeBarardinis[29] focused attention on the disease as an entity with a review of 16 cases collected from the literature. Although Page and Good[27] remarked in 1957 that only 23 well documented cases could be found in the literature, Becker, et al.[4] in 1959 reported in some detail on 33 cases and added two additional ones. With the case of Gorlin and Chaudhry,[15] a total of 36 patients with the disease will have been described in the literature prior to the time of this publication. Oral roentgenograms of an additional patient appear in the textbook by Shafer et al.,[34] however, there is no accompanying documentation of the case. There are three additional cases described in the literature which perhaps should be included. One is not typical;[3] one was given the name "Chronic Familial Neutropenia" even though a periodic recurrence of the neutropenia was clearly described;[20] and the third[36] appears to have been missed by reviewers previous to Gorlin and Chaudhry.[15]

Rather complete reviews of the limited literature are available[4, 10, 27, 29] and several authors have prepared tables in which each patient is listed and commented upon regarding his sex and age,[4, 15, 27, 29] associated symptoms,[4, 27, 29] bone marrow findings,[4, 27] oral ulcerations,[15] and treatment.[4, 27]

Nineteen of the 36 cases in the literature occurred in females. The onset of associated symptoms, which usually preceded a confirmation of the diagnosis, frequently took place in infancy and was recognized before the age of ten years in 31 of the patients. In four patients[13, 14, 24, 33] the disease apparently initiated after age 40 and in one[24] first symptoms appeared at the age of 76. In all patients except two, there was no remission of the condition once it was recognized.

Serial bone marrow studies by some workers revealed a complete suppression of the granulocytic series of cells during the neutropenic period of the cycle.[6, 14, 24, 29, 38] Other investigators observed only a maturation arrest at the promyelocyte level.[17, 25, 26, 43] During the intervals between the neutropenia, the bone marrow is normal.

There is lack of agreement in regard to associated laboratory findings. Blood platelet concentrations have been reported as increased[11, 12] and decreased.[32] Elevated sedimentation rates have been described in only three patients.[5, 6, 31] There was a coincidental finding of lymphoma in two patients.[25]

Thompson,[37] Jackson et al.,[18] and Stephens and Lawrence[36] felt that the

*Associate Professor of Oral Medicine and Director of Periodontics, University of Pennsylvania, School of Dentistry.

**Associate Professor of Oral Medicine and Director of Oral Diagnosis, University of Pennsylvania, School of Dentistry.

TABLE 1

Periodontitis Shown by Roentgenograms	Periodontitis Described	Gingivitis Described	
Levine [20]	Cobet [7]	Becker [4]	Imerslund [13]
*Page [27]	Coventry [8]	Borne [5]	**Kitchen [19]
Shafer [34]	Owren [26]	Doan [9]	Magnin [23]
		Gorlin [15]	Reznikoff [30]
		Hahneman [16]	Rutledge [31]
		Stephens [36]	

*Roentgenographic changes in this patient described by Gorlin [15]
**Same patient

neutropenic and menstrual cycles were related in the disease, however, other authors denied such a relationship.[14, 17, 29] Pregnancy was reported to improve the symptoms in one patient[18] and to make them worse in another.[1] One patient was reported to receive benefit after menopause had been surgically induced.[36]

The etiology of cyclic neutropenia is unknown. Possible relationships between the disease and hypersplenism,[6, 8] endocrine dysfunction,[24, 37] allergy,[6, 29] reticuloendothelial dysfunction,[25] and heredity[8] have been discussed. The clinical picture is confused by the appearance of most any inflammatory or infectious process to which the patient may be susceptible or exposed during the period of decreased resistance associated with the neutropenia. Therapeutic measures have of necessity been aimed at control of the accompanying disease processes. Attempts to treat the primary disease have been generally unsuccessful. Although Monto[24] reported a beneficial effect of corticotropin therapy, this was not supported by the results of others.[6, 25, 29] Splenectomy was performed in 13 cases with some therapeutic effect if the patient was over 25 and had splenomegaly.[4, 14, 24, 33] In no case did outright cure of the disease result.

Coventry[8] states that to qualify for the diagnosis of cyclic neutropenia a patient must have a history of recurrent infection over a period of years, have recurrent cycles of neutropenia during the infections, and have good health in spite of the infection and neutropenia. It is interesting that the most commonly described clinical manifestation of the disease is that of oral ulcerations occurring concomitantly with the neutropenia. Gorlin and Chaudhry[15] have recently emphasized this observation.

In the report of individual cases, mention was frequently made of dental problems, other than oral ulcers. Most cases have been reported by physicians and work up of their patients has not included a detailed oral examination or intra-oral roentgenograms. It is apparent, however, that involvement of the periodontal structures may be a feature of this disease. Table 1 reveals three reports in the literature in which patients with cyclic neutropenia have been shown by roentgenograms to have extensive loss of alveolar and support-

Fig. 1. Case I—Photograph of four year old boy (M.L.) Note the heavy stain and calculus formation. Gingival inflammation is noted about the teeth.

Fig. 2. Photograph of the right posterior area of case I. Recession of the gingival margin into the bifurcation of the mandibular deciduous first molar is seen.

Fig. 3. Photograph of the left posterior area of case I. The rolled thickened gingival margin is present in the maxilla and the mandible.

ing bone; in three cases alveolar process destruction is strongly indicated by such comments as: "teeth had to be extracted due to purulent gingivitis"; in 10 cases evidence suggesting periodontal involvement was limited to a mention of gingivitis. The gingivitis was sometimes qualified as being chronic, persistent, hemorrhagic or hypertrophic. In the remaining cases, the state of health of the periodontal tissues or teeth was not commented upon.[1-3, 6, 10, 12-14, 18, 21, 23-25, 29, 31, 33, 35, 37, 38]

Three additional cases of cyclic neutropenia are described below. Represented are patients with deciduous, mixed and young adult dentitions. A striking feature of each case was the involvement of the periodontal tissues.

CASE I

A 4 year old white male (M.L.) who had been troubled with canker sores on the tongue, lips and cheeks since infancy and had gingival bleeding since the age of 3 years was first seen in 1959.

The medical history revealed the presence of a heart murmur since early life and at the age of six months he was operated on for rectal abscesses. Colic was present during the first year. The child had frequent nosebleeds and was diagnosed as being anemic at age 3½ years.

The remainder of his system review was essentially negative.

The mother had a history of recurrent canker sores and there are no siblings.

The gingival tissues were red and swollen and gingival pockets were noted throughout the mouth (Figs. 1, 2 and 3). Pocket

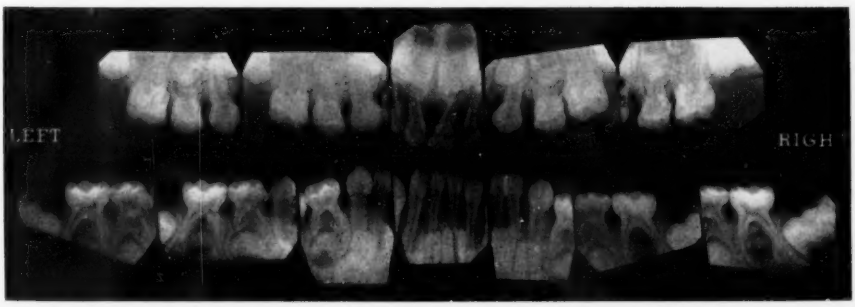


Fig. 4. Full mouth radiographs of case I. The horizontal resorption of the alveolar and supporting bone is seen throughout the mouth.

depth extended into the bifurcations of the mandibular deciduous molars and the maxillary first deciduous molars. Slight mobility of the teeth was present. The radiographs (Fig. 4) revealed horizontal resorption of the alveolar and supporting bone throughout the mouth. The formation of the permanent teeth appeared normal on the radiographs.

The tonsils were huge but not infected. Moderate enlargement of the cervical and axillary lymph nodes was noted. A moderately loud, rough systolic murmur was heard over the right upper chest, posteriorly.

The following studies were performed in Children's Hospital of Philadelphia.

TABLE 2

	August 18, 1959	August 21, 1959
Hemoglobin	10.8 gms. %	10.0 gms. %
White Blood Cells	8250	4900
Neutrophils	6%	9%
Eosinophils	9%	7%
Urine—Specific Gravity	1.018	1.018
Blood Chemistry		
Albumin	5.1	5.1
Globulin	3.2	3.2
Urea Nitrogen	25 mgm. %	
Fasting Blood Sugar	45 mgm. %	

It was the impression of some of the observers that chronic lymphocytic leukemia was a possible diagnosis but upon review of the blood smear and marrow biopsy Dr. Irving J. Wolman, Chairman of Hematology, felt that this would be a premature diagnosis. In any case, it appeared that the patient's gums and blood conditions were related and that his gingival infection was unlikely to heal due to the presence of a marked diminution in neutrophile leukocytes.

The patient was placed on prophylactic penicillin 200,000 units twice daily in an effort to limit the infection which was present.

Dr. Robert Kaye, pediatrician at Children's Hospital, examined the patient and suggested serial differential counts weekly for three weeks in order to rule out the possibility of cyclic neutropenia.

Studies were repeated in October 1959 with the following results:

The peripheral blood showed 350,000 Platelets, 0.7% Reticulocytes, Wbc 3500 —14% of which were Neutrophils, 1% Eosinophils, 61% Lymphocytes and 24% Monocytes. His hemoglobin was 10.0 mg.%. A bone marrow aspiration was done and the specimen was normal in cellularity. The cells of the granulocytic series were very scanty. The lymphocytic series was remarkably active. Most of the lymphocytes were small forms presenting very scanty cytoplasm. Erythropoietic activity was normal and thrombopoiesis was adequate. No abnormal cells were seen.

The summary of the findings at Children's Hospital were as follows:

"Dr. Wolman understood from the mother that the child had recurrent bouts of low grade fever. We thought that we could use the tag of periodic syndrome or periodic disease as the diagnosis of the condition of the child for failure of finding better terms. We do feel that the child has lymphocytic hyperplasia or depression of the myelopoietic series but we do not think that the child has leukemia."

CASE II

This seven year old white female (B.C.) was brought to the oral diagnosis clinic in March 1960 with a chief complaint of recurrent ulcerations of the mouth. The history revealed that one to several ulcers had occurred at three to four week intervals since the patient was 16 months of age. The lesions rarely bled and remained for a period of about 10 days. The cycle appeared to be about 25 to 30 days and the oral symptoms were occasionally accompanied by fever. Both parents were living and well and two siblings showed no evidence of this condition. Though there were no oral ulcers at the initial examination, we were impressed with the condition of the gingivae.

Fig. 5. Case II—Photograph of seven year old girl (B.C.) The inflamed thickened gingival tissues are seen about the anterior teeth.

Fig. 6. Photograph of the left posterior area of case II. Recession of the gingival margin about the deciduous molar is seen.

There was a generalized enlargement and a bluish red color with an increased tendency to hemorrhage upon probing (Figs. 5, 6 and 7). The mother noted that there was some bleeding with toothbrushing. Full mouth roentgenograms (Figs. 8 and 9) revealed resorption of the bone between the roots of the deciduous mandibular molars.

The following studies were performed:

TABLE 3

	March 1	March 8	March 20	April 5
			Admitted to Children's Hosp. of Phila.	Discharged
Hemoglobin	11.3 gm.	11.9 gm.	10.4 gm.	
WBC	4000	4700	3650	
Neutrophiles	2%	28	4	54%
Seg.	1%	18	–	
Non Seg.	1%	10	1	
Eosinophiles	2%	8%	3%	
Basophile	2%	0	1	
Lymphocytes	70%	55%	40%	
Monocytes	1%	9%	4%	

Blood chemistries BUN 10 mg%, blood sugar 84 mg %, serum protein electrophoresis revealed the following findings: albumin 52.5%, 2.19; alpha 1, 3.1% 0.44; alpha 2, 14.0%, 0.95; beta 18.5%, 1.25; gamma 29.0%, 1.97; the serum albumin was somewhat low and the gamma globulin was moderately increased. Urinalysis, showed no abnormalities. Nasopharyngeal culture showed a heavy growth of H. influenziae but no other significant pathogens. Tuberculin Test 1/1000 was negative. Oral ulcers developed after the initial examination and prior to admission to Children's Hospital of Philadelphia. By March 28 when her neutrophil count had risen to 17%, her oral lesions were much improved. Bone marrow examination revealed depressed myelopoiesis and especially maturation arrest with some toxic changes, slight eosinophilia, histiocytosis, marked normoplastic marrow. The leukocytes showed no evidence of auto-agglutination, nor did her serum agglutinate a panel of leukocytes from 6 normal donors.

Dr. Robert Kaye of Children's Hospital suggested treating the patient with penicillin when her neutrophils fell below 30%.

The diagnosis of cyclic neutropenia was established.

In June of 1960 the deciduous left maxillary 1st and 2nd molars exfoliated and the gingival tissues continued to be puffy red and retractable. A mixture of equal amounts of lidocaine hydrochloride ointment and an emollient adhesive ointment* was prescribed for application to the oral ulcers.

*Orabase—Squibb.

CASE III

A 16 year old white female (D.S.) presented herself because of loose teeth and inflamed gingivae of 3 years duration. The patient was aware that the maxillary and mandibular incisors were drifting (Fig.

Fig. 7. Photograph of the right posterior area of case II. Inflammatory changes involving the gingiva are seen in the deciduous molar region.

Fig. 8. Full mouth radiographs of case II. Note the interradicular resorptive lesions involving the deciduous mandibular second molars.

Fig. 9. Lateral jaw radiographs showing the alveolar resorption in case II.

Fig. 10. Case III—Photographs of a sixteen year old girl (D.S.) The red enlarged gingival tissues are seen about the maxillary left central incisor and the maxillary right cuspid.

TABLE 4

Lab studies		10/9/57	12/11/57	12/14/57
Blood	Hemoglobin	14.2	15	
	Hematocrit	41 vol %	45	
	White Blood	5000	5,050	9000
	Poly-segs	65	12	50
	Band Forms	6	8	10
	Lymphocytes	15	65	27
	Monocytes	13	24	4
	Eosinophils	1	2	
	Basophils			1
	Platelet count	84,000		
	Glucose	91	101	
	Blood Urea Nitrogen	4	16	
Urine				
	Appearance	straw	yellow	straw
	Reaction	acid	acid	acid
	Specific Gravity	1.016	1.020	1.027
	Sugar	negative	negative	negative
	Albumin	—	—	—
	Occult Blood	—	—	—
	r.b.c. per hpf	occ	occ	0
	w.b.c. per hpf	0-1	4-5	1-2
	Cast	—		
	Epith	0-8		

10). Full mouth roentgenograms (Fig. 11) revealed severe resorption of the alveolar process affecting the anterior teeth. Because of the suspicion of an extraoral influence, the patient was referred to her physician for a complete medical evaluation. The patient did not keep her appointment with the physician and was seen one year later at which time the anterior teeth exhibited increased mobility and the gingival inflammation was still present. The patient was again referred to her physician who admitted her to Lankenau Hospital of Wynnewood, Pa. on 10/9/57 for study (See Table 4). Complete skeletal radiographs were negative except for the films of the alveolar process. The patient was discharged on 10/18/57 with a final diagnosis of Agranulocytosis. The patient was readmitted to Lankenau Hospital on 12/4/57 because of a loss of 12 pounds in several weeks. She was discharged on 12/16/57 with a final diagnosis of Agranulocytosis.

She was placed on a high protein diet and given bone extract. The patient was re-admitted to the hospital on 7/23/58 with a chief complaint of sore throat of one week duration. Dr. John Hodges saw the patient in consultation and diagnosed the condition as cyclic neutropenia. Since her blood picture was improved, it was decided that a

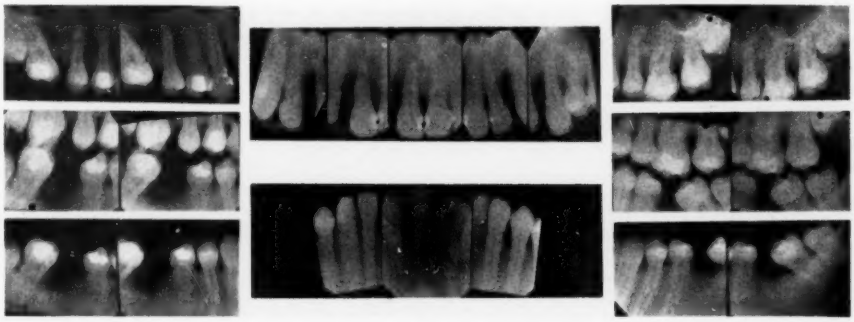


Fig. 11. Full mouth radiographs of case III. The advanced resorptive lesion of the alveolar process is seen about the maxillary and mandibular left central incisors, the maxillary right cuspid and the maxillary right posterior teeth.

tonsillectomy would not have an adverse effect upon the disease. This was performed on July 29th and healing was uneventful.

The following lab studies were performed during this hospital stay. Table 5.

TABLE 5

	7/24/58	7/30/58	11/3/58
Hemoglobin	15.9	13.4	15.09
Hematocrit	43 vol %	42 Vols %	46%
White Blood Count	7400	6800	4800
Poly-segs	60%	66%	
Lymphomaytes	4%	1%	
Monocytes	36%	21%	61 %
Eosinophils	1%	3%	1 %
Platelet Count	1%	1%	200
Blood Urea Nitrogen	284,000		200,000
Uric Acid	7 mg %		
	4.5 mg %		

On 11-3-58 the poly-segs. were 34%.

A rather striking reduction in the gingival inflammation was observed after this hospitalization (Fig. 12). Full mouth radiographs taken at this time demonstrated interesting changes in the interdental areas (Fig. 13).

Fig. 12. Case III—This picture was taken two years after Figure 10. No dental treatment was performed during this time other than shortening the maxillary left central incisor. Note the reduction in gingival inflammation in the anterior region.

DISCUSSION

These three cases present an interesting picture of the periodontal manifestations associated with the systemic alteration of cyclic neutropenia. In Case I we observe the unusual finding of resorption of the alveolar process in the deciduous dentition from periodontal disease.

Case II exhibits resorption of the bone about the deciduous teeth but no radiographic changes involving the recently erupted permanent teeth.

Case III shows extensive resorption of the alveolar process about the permanent teeth. The gingival inflammation in all three cases was marked and characterized by hyperemic, enlarged and detached tissues about the teeth. When comparing Case 3 with the one cited by Shafer, Hine and Levy[34] and the one reported by Gorlin and Chaudhry[15] there appears to be a marked similarity in the pattern of bone loss in the mandibular incisor region. There is advanced vertical resorption of the alveolar and supporting bone about the mandibular incisors in Case III as well as the two[34, 15]

Fig. 13. Full mouth radiograph of case III taken two years after Figure 11. Note the formation of a cortical crest in the maxillary right cuspid and posterior areas as well as the mandibular left central incisor region.

cited above. We intend to follow these children closely and determine if there is loss of the alveolar process in cases 1 and 2 following eruption of the permanent teeth.

Consideration of the ages of the patients and the type of periodontal breakdown influences the clinician to classify these three cases as periodontosis coupled with periodontitis. Review of the literature indicates that periodontosis without a concomitant gingival inflammation is extremely rare if not non-existent. Therefore, we believe that these cases should be classified in the category of periodontosis. It does appear from recent reports that the entity of periodontosis is being subdivided as more information is learned about the patients afflicted by the condition. Ingle[39] in his review and report of a new case of Papillon-Lefevre syndrome with periodontosis points out the high incidence of gingival inflammation in previously documented cases. Schluger's case portrayed in Ingle's article clinically resembles the cases reported in this paper.

It becomes obvious that a single blood count is inadequate if the clinician is considering cyclic neutropenia. Hematologic studies should be repeated weekly for four successive weeks in order to determine if there is a cyclic nature to the disease. If this were done routinely, we suspect that this entity would not be as rare as it now appears.

Though the prognosis of the neutropenic condition is not encouraging, we are attempting to maintain high standards of oral hygiene in these cases. Periodic scaling and polishing is performed and the patients have been instructed in the techniques of oral physiotherapy.

It is also interesting to note that the oral manifestations of mouth ulcers and periodontal disease were responsible for the patient seeking dental care, and then through systemic evaluation, it was determined that cyclic neutropenia was present. In patients with recurrent oral ulcers and marked alveolar bone loss, weekly blood studies to rule out cyclic neutropenia seem indicated. The dentist may have the first opportunity to diagnosis the disease.

REFERENCES

1. Barling, B.: Chronic Cyclical Granulopenia. Proc. Roy. Soc. Med., 41:653-654, 1948.

2. Barsby, B. E. and Close, H. G.: Recurrent Neutrophil Agranulocytosis. Lancet, 1:99-101, 1942.

3. Barton, G. M. G.: Recurrent Agranulocytosis: Report of a case. Lancet. 254:103, 1948.

4. Becker, F. T., Coventry, W. D., and Tuura, J. L.: Recurrent Oral and Cutaneous Infections Associated with Cyclic Neutropenia. A.M.A. Arch. Dermat., 80:731-741, 1959.

5. Borne, S.: Cyclic Neutropenia in Infant. Pediatrics, 4:20-78, 1949.

6. Chaiken, B. H.: The Effect of ACTH in Periodic (Cyclic) Neutropenia. Blood, 9:83-89, 1954.

7. Cobet, R., and Schilling, V.: Periodisch-rezidivierende Neutropenie mit Monocytose. Folia Haemat., 70:286-304, 1951.

8. Coventry, W. D.: Cyclic Neutropenia: Report of a Case Treated by Splenectomy. J.A.M.A. 153: 28-31, 1953.

9. Doan, C. A.: The Neutropenic State J.A.M.A., 99:194-209, 1932.

10. Duane, G. W.: Periodic Neutropenia. A.M.A. Arch. Int. Med., 102:462-467, 1958.

11. Embleton, D.: Rhythmical Agranulocytosis. Brit. Med. J., 2:1258-1259, 1936.

12. Embleton, D.: Rhythmical Neutropenia with Recurrent Buccal Ulceration. Proc. Roy. Soc. Med., 30:980-982, 1937.

13. Erf, L. A., and Dry, K. E.: Primary Splenic Neutropenia: Case Report. Amer. J. Clin. Path. 19:48-50, 1949.

14. Fullerton, H. W. and Duguid, H. L. D.: A Case of Cyclical Agranulocytosis with Marked Improvement Following Splenectomy. Blood, 4:269-277, 1949.

15. Gorlin, R. J. and Chandhry, A. P.: The Oral Manifestations of Cyclic (Periodic) Neutropenia. Does Peradenitis Mucosa Necrotica Recurrens (Sutton) Exist? A Need for Evaluation. A.M.A. Arch Dermatol., In Press.

16. Hahneman, B. M. and Alt, H. L.: Cyclic Neutropenia in a Father and Daughter. J.A.M.A. 168:207-272, 1958.

17. Inerslund, Olga: A Case of Cyclic Agranulocytosis. Acta. Paediat., 30:232-250, 1942.

18. Jackson, H., Merrill, D., and Duane, M.: Agranulocytic Angina Association with the Menstrual Cycle. New Eng. J. Med., 210:175-176, 1934.

19. Kitchen, P. C.: Oral Observations in the Case of Periodic Agranulocytosis. J. Dent. Res. 14:315-322, 1934.

20. Levine, Sydney: Chronic Familial Neutropenia with Marked Periodontal Lesions. Oral Surg., Oral Med., Oral Path., 12:310-314, 1959.

21. Loffler, W., and Maier, C.: Uben einen Fall von Feltzshem Syndrom mit Cyclischer Agranulocytose: Zugleich ein Beitrag zur Auffassung des Infektiosen Rheumatismns als Ausdruck der Individuellen Reaktion. Cardiologia, 12:195-210, 1947-1948.

22. Magnin, G. E., Suits, J. J., and Wenzel, F.: Cyclic Neutropenia. Report of a Case treated with Splenectomy. Wisconsin Med. J., 192-195, 1957.

23. Moncrieff, A.: Recurrent Neutropenia. Arch. Dis. Child., 26:438-440, 1951.

24. Monto, R. W., Shafer, H. C., Brennan, M. J. and Rebuck, J. W.: Periodic Neutropenia treated by Adrenocorticotropic Hormone and Splenectomy. New Eng. J. Med. 246:893-896, 1952.

25. Natelson, R. D.: Cyclic Neutropenia with Giant Follicular Lymphoblastoma and Lymphosarcoma Blood, 8:923-933, 1953.

26. Owren, P. A.: Cyclic Agranulocytoses. Acta Med. Scand., 134:87-97, 1949.

27. Page, A. R. and Good, R. A.: Studies on Cyclic Neutropenia: A Clinical and Experimental Investigation. A.M.A. J. Dis. Child., 94:623-661, 1957.

28. Page, A. R. and Good, R. A.: A Clinical and Experimental Study of the Function of Neutrophils in the Inflammatory Response. Amer. J. Path., 34:645-669, 1958.

29. Reimann, H. A. and DeBarardinis, C. T.: Periodic (Cyclic) Neutropenia, An Entity: Collection of Sixteen Cases. Blood, 4:1109-1116, 1949.

30. Reznikoff, P.: Cyclical Neutropenia: A Case with Bone Marrow Findings before and after Splenectomy. Trans. Assoc. Amer. Phys., 59:276-281, 1946.

31. Rolland, C. F. and Davidson, L. S. R.: A Case of Cyclic Neutropenia. Edinburgh Med. J. 57:205-210, 1950.

32. Rutledge, B. H., Hansen-Pruss, O. C. and Thayer, W. S.: Recurrent Agranulocytosis. Bul. John Hopkins Hosp. 46:369-389, 1930.

33. Sandella, J. F.: Cyclic Acute Agranulocytosis: Report of a case with Improvement after Splenectomy. Ann. Int. Med., 35:1365-1371, 1951.

34. Shafer, W. G., Hine, M. K., and Levy, B. M.: A Textbook of Oral Pathology, Saunders, Phila., 714p. 1958, p. 588.

35. Siguier, F., and Sabaoum, J.: Maladie Periodique avec Crises de Sialharrhic et Neutropenie Cycliques. Bull. Mem. Soc. Med. Hop. Paris 70:27-31, 1954.

36. Stephens, D. J. and Lawrence, J. S.: Cyclical Agranulocytic Angiva. Am. Int. Med. 9:31-37, 1935.

37. Thompson, W. P.: Observations on the Possible Relation Between Agranulocytosis and Menstruation with Further Studies on a Case of Cyclic Neutrooenia. New Eng. J. Med., 210:176-178, 1934.

38. Vahlquist, B.: Cyclic Agranulocytosis Acta Med. Scand. Suppl. 170:531-542, 1946.

39. Ingle, J.: Papillon-Lefevre Syndrome: Precocious Periodontosis with Associated Epidermal Lesions. J. of Periodontology 30:230-237, 1959.

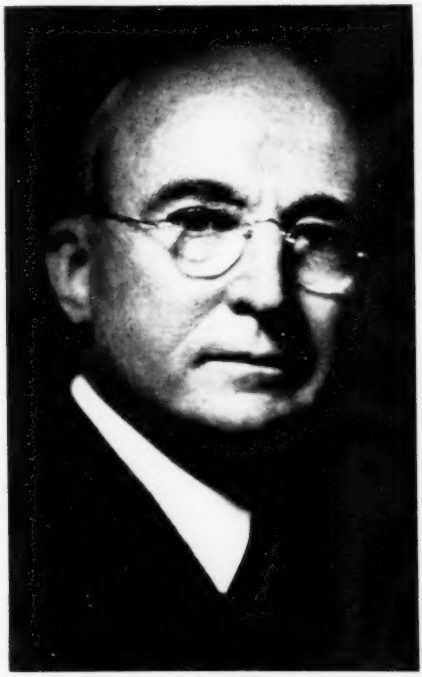
SCHOOL OF MEDICINE, BOSTON UNIVERSITY

The Department of Stomatology, Division of Graduate Studies, School of Medicine, Boston University and the Massachusetts Memorial Hospitals, will present an Advanced Course in Periodontal Therapy by Drs. Henry M. Goldman, Bernard S. Chaikin, Gerald M. Kramer, William Pendergast and Associates, April 24 to 29, 1961.

For further information write Director, Postgraduate Courses, Department of Stomatology, Massachusetts Memorial Hospitals, 750 Harrison Avenue, Boston 18, Massachusetts.

Dr. Arthur H. Merritt

May 2, 1870 - February 9, 1961



Dentistry lost one of its most outstanding members when Dr. Arthur H. Merritt, Riverdale-on-Hudson, New York, died on February 9, 1961, at the age of 90. His contributions to dentistry, particularly in periodontology and dental journalism, were so noteworthy that the July 1959 issue of this Journal was dedicated to him. In that issue are published many testimonials from his friends regarding his many meritorious contributions to dentistry and his unwavering dedication to the best in dental science.

Dr. Merritt was awarded honorary degrees by the University of Michigan, Columbia University, University of Maryland, New York University and Hartwick College.

Dentistry is better because of Dr. Merritt's activities and his influence for good will be sorely missed.

Announcements . . . .

ANNUAL MEETING, 1961

The American Academy of Periodontology will hold its 47th anniversary meeting aboard the M. S. Bergensfjord, sailing from Philadelphia, Pennsylvania, October 20, 1961, at 11:00 A.M., to Bermuda, and returning to Philadelphia on October 25, 1961.

The Caribbean Cruise Lines advise us that the reservations for the Bermuda cruise are coming in very satisfactorily. All Academy members who are contemplating making the trip, please get your request in immediately. After February 15, announcements were sent to other periodontal groups throughout the country, and if the ship is not filled by April 1, we will accept applications from American Dental Association members.

The rates for the cruise range from $145 to $335 per person which includes stateroom, meals, and recreation aboard ship. For further information write: Caribbean Cruise Lines, Mr. E. M. Baskette, Southern Regional Manager, 301 Roper Building, Miami 32, Florida.

A.A.P. GOLF TOURNAMENT

In connection with the Bermuda cruise meeting, a golf tournament has been arranged for Sunday, October 22, 1961. There will be divisions with prizes for all kinds of golfers from the duffer to the very best. There will be divisions for those members who have attested club handicaps. Players without club handicaps will play with the Calloway System which is a great equalizer. Greens fees are $5. The usual caddie fee is $3.50 for a single and $5.60 for a double caddie. Golf clubs are available for rent from $2.50 to $3.50 so that those who fly to Philadelphia need not bring their own clubs. Transportation at a minimal rate will be arranged from the ship to the course. If there are sufficient women who indicate an interest in playing, a tournament may be arranged for them. Such an effort, of course, will depend on the response from the ladies. All interested should write Dr. Arthur Gold, 26 Maple Street, Springfield, Massachusetts, for details.

SOUTHERN ACADEMY OF PERIODONTOLOGY

The Southern Academy of Periodontology will hold its annual meeting on June 11, 12 and 13 at the Riviera Motel in Atlanta, Georgia. Dr. Henry Goldman from Boston and Dr. Frank Beube from Columbia University will be the principal clinicians. Four limited attendance clinics will be given by Academy members. A truly great periodontics seminar has been timely programmed with an abundance of recreational and social activities for all the family at the exquisite Riviera Motel. A.D.A. members are welcomed as guests.

For further information please write Dr. Jesse J. Morris, Secretary, 101 Third Street, North East, Atlanta, Georgia.

PROGRAM FOR THE SPRING SEMINAR OF THE AMERICAN ACADEMY OF PERIODONTOLOGY

Ann Arbor, Michigan—April 24-26, 1961

Monday, April 24

A. M.

9:30 - 12:00 WOUND AND BONE HEALING. William G. Shafer, Indianapolis, Indiana, Professor and Chairman of the Department of Oral Pathology, School of Dentistry.

P. M.

1:30 - 4:30 CURRENT CONCEPTS OF IMMUNOLOGY. Walter J. Nungester, M.D., Ann Arbor, Professor and Chairman Department of Bacteriology, The University of Michigan Medical School.

Tuesday, April 25

A. M.

9:00 - 12:00 STOMATITIS. Donald A. Kerr, Ann Arbor, Professor and Chairman, Department of Oral Pathology and Periodontia, The University of Michigan School of Dentistry.

P. M.

1:30 - 4:00 APPLICATIONS OF PSYCHIATRY TO DENTAL PRACTICE. Leonard E. Himler, M.D., Ann Arbor, Associate Professor of Mental Health, School of Mental Health, The University of Michigan.

6:00 SOCIAL HOUR AND DINNER. Michigan Union.
"PROJECT HOPE." Ralph C. Charbeneau, Detroit, Director of Public Relations, Ex-Cell-O Corporation, Detroit, Michigan.

Wednesday, April 26

A. M.

9:00 - 12:00 THE PRESENT STATUS OF CONSERVATIVE PERIODONTAL THERAPY AND REATTACHMENT. Erwin M. Schaffer, Minneapolis, Professor and Chairman of the Department of Periodontics, School of Dentistry, University of Minnesota.

P. M.

1:30 - 3:30 THE PRESENT STATUS OF BONE SURGERY IN PERIODONTAL THERAPY. Frank M. Wentz, Chicago, Professor of Periodontics, School of Dentistry, Loyola University.

PACIFIC COAST ORAL PATHOLOGY ASSOCIATION

The Pacific Coast Oral Pathology Association announces the annual Oral Pathology Workshop, Conference and Seminar on April 15, 1961, at the Letterman General Hospital, San Francisco, California. The theme will be "Disturbances of the Salivary Glands." Moderator will be Dr. S. N. Bhaskar, A.F.I.P., and the Program Chairman is Dr. Daniel A. Collins. The fee is $20.00.

For further information write Dr. R. Gordon Agnew, Executive Secretary, Pacific Coast Oral Pathology Workshop, University of California Medical Center, San Francisco 22, California.

1961 OFFICERS OF CALIFORNIA ACADEMY OF PERIODONTOLOGY

First row left to right: B. O. A. Thomas, First Vice President; Max Rabinovich, Second Vice President; Philip S. Haley, Chairman Research Committee; Clarence E. Butler, Treasurer; Mario Pieri, Third Vice President.

Second row, left to right: J. James Pfister, Immediate Past President; Antonio R. Sanchez-Corea, Editor; Kent F. Kohler, Assistant Editor; Lowell N. Peterson, Historian, Librarian; Robert L. Roseberry, Secretary, Academy Review; Robert E. Oslar, President; V. John Oulliber, Business Manager, Academy Review; Elmer G. Smith, Financial Secretary; Albert Wasserman, Secretary. Stanley S. Sepich, Recording Secretary, not in picture.

The California Academy of Periodontology Second Spring (May) Conference will be held in Reno, Nevada, Friday, Saturday and Sunday, June 2, 3 and 4, 1961. There will be lectures, clinics in periodontics; entertainment and tours for the wives while the members are in scientific sessions. For further information write Dr. B. O. A. Thomas, Program Chairman, 668 Homer Avenue, Palo Alto, California.

UNIVERSITY OF ALABAMA SCHOOL OF DENTISTRY

The University of Alabama School of Dentistry announces the following course:

April 15 and 16—Management of Advanced Periodontal Disease:
John F. Prichard, D.D.S.

For further information write the University of Alabama School of Dentistry, Birmingham, Alabama.

TUFTS UNIVERSITY SCHOOL OF DENTAL MEDICINE

Tufts University School of Dental Medicine announces the following postgraduate courses:

April 24 through 28, 1961—A special participation course in periodontology, conducted by Irving Glickman, Professor of Oral Pathology and Periodontology and staff. Tuition will be $175.00 and the class is limited.

In this course emphasis is placed upon learning by clinical experience in addition to lectures and demonstrations. Half the time is devoted to clinical sessions in which each participant treats patients under Dr. Glickman's guidance. Bifurcation, involvement, infrabony pockets, the "unembellished gingivectomy," frenectomy and splinting are dealt with at the chairside. The "Bone Factor" concept for the diagnosis, determination of prognosis and treatment planning for individual teeth and the dentition as a functioning unit will be explained in detail.

May 23-24, 1961—The Diagnosis and Treatment of Pain and Other Symptoms Arising from Temporomandibular Joint Disturbances, conducted by Dr. Harmon Shohet, former instructor, Harvard University Dental School. Tuition is $75.00 and the class is limited.

In this course Dr. Shohet will explain how to diagnose problems originating from temporomandibular joint disturbances and how to correct them. It will be a practical course in which the management of actual cases will be explained.

October 9-13, 1961—A Special Participation Course in Periodontology, conducted by Dr. Irving Glickman and Staff. Tuition is $175.00 and the class is limited.

In this course emphasis is placed upon learning by clinical experience in addition to lectures and demonstrations.

For further information write Director of Division of Graduate and Postgraduate Studies, Tufts University School of Dental Medicine, 136 Harrison Avenue, Boston 11, Massachusetts.

ANNUAL BERKSHIRE CONFERENCE

Major General Joseph L. Bernier, Assistant Surgeon General and Chief of the Dental Corps, U. S. Army, will be one of the principal speakers at the Tufts 12th Annual Berkshire Conference in Periodontology and Oral Pathology. General Bernier will present a clinicopathological seminar on the subject, "Developments in the Understanding of Malignancy as They Relate to the Diagnosis and Treatment of Oral Lesions."

The Berkshire Conference, now in its 12th year, will be conducted at Eastover in Lenox, Massachusetts, June 18-22, 1961. The overall theme of the conference this year is "The Current Clinical Significance of Developments of the Past Twelve Years." Other Lecturers on the Conference faculty will be Finn Brudevold, Irving Glickman, Reidar F. Sognnaes, John B. MacDonald, Sigurd Ramfjord, Everett Shapiro, David Weisberger, and Frank M. Wentz.

The conference is open to general practitioners and dentists in specialized practice. Information regarding participation may be obtained by writing Tufts Berkshire Conference, Tufts University School of Dental Medicine, 136 Harrison Avenue, Boston 11, Massachusetts.

THE MIDWEST SOCIETY OF PERIODONTOLOGY

Henry M. Swenson presenting the Past President's Plaque to Erwin M. Schaffer in recognition of his service as President of the Midwest Society of Periodontology.

The Midwest Society of Periodontology Annual Scientific Session was held at the Blackstone Hotel in Chicago on February 5, 1961. The program was enthusiastically received with over 100 members and guests attending. The following officers were elected: Henry M. Swenson, President; D. P. Nachazel, President-Elect; Luke Howlett, Secretary; Marmion Houglum, Treasurer.

UNIVERSITY OF PENNSYLVANIA

The University of Pennsylvania School of Dentistry announces that Dr. Lewis Fox and Associates will present a three day course, May 3 to 5, 1961 on Principles of Occlusion. This course is dedicated in its purpose to fulfill the need for presenting the fundamentals of occlusion in relation to the practice of all dentistry. It is prepared to meet the needs of both general practitioner and specialist. The entire course will be directed toward correlating the principles of occlusion to clinical criteria and therapeutic endeavors.

For further information write Postgraduate Courses, School of Dentistry, University of Pennsylvania, 4001 Spruce Street, Philadelphia 4, Pennsylvania.

UNIVERSITY OF PENNSYLVANIA
RESEARCH AND TRAINING TRAINEESHIPS

Research and Teaching Traineeships in the basic sciences, in periodontology and in the comprehensive treatment and rehabilitation of the cleft palate patient are available at the School of Dentistry, University of Pennsylvania.

The traineeship in periodontology is a 2 year program devoted to basic science studies, clinical practice and research. It is intended primarily for those individuals who wish to enter into teaching and research in periodontology.

All traineeships are supported by the U. S. Public Health Service. Annual stipends vary from $4,500 and up depending on the qualifications and needs of the successful candidates. Inquiries for the basic science and cleft palate traineeships should be sent to the Chairman, Committee on Traineeships and Fellowships, University of Pennsylvania, School of Dentistry, 4001 Spruce Street, Philadelphia 4, Pennsylvania. Inquiries for the traineeships in periodontology should be sent to Dean, Graduate School of Medicine, 237 Medical Laboratories, University of Pennsylvania, Philadelphia 4, Pennsylvania.

ALBERT EINSTEIN COLLEGE OF MEDICINE

Albert Einstein College of Medicine, Yeshiva University, announces postgraduate courses in periodontia to be presented during the spring of 1961. These courses are designed to be integrated into the general dental practice. Lecture sessions will be held at the Albert Einstein College of Medicine and the clinical demonstrations at the Dental Clinic, Jacobi Hospital.

For further information and application, please write to Director, Postgraduate Dental Division, Albert Einstein College of Medicine, Eastchester Road and Morris Park Avenue, Bronx 61, New York.

UNIVERSITY OF PENNSYLVANIA

Applications are being accepted for a Clinical Fellow in Oral Pathology supported by the American Cancer Society, tenable at the Department of Pathology, School of Dentistry, University of Pennsylvania, for a period of one year beginning July 1, 1961. This fellowship is open to U. S. citizens who are graduates of a U. S. dental school. A stipend of $3600 (tax free) accompanies the fellowship. The program is planned to suit the specific needs and interests of the Fellow. Each candidate should submit a curriculum vitae and letters of reference. Please reply to Dr. C. E. Wilde, Jr., Department of Pathology, School of Dentistry, University of Pennsylvania, 4001 Spruce Street, Philadelphia 4, Pennsylvania.

BALINT ORBAN MEMORIAL SEMINAR

The Rocky Mountain Dental Seminar will hold its second session, in memory of its founder, Dr. Orban, in Boulder, Colorado, June 25th to 28th, 1961. Theme for the Seminar is "Clinical and Microscopic Pathology of the Periodontium and Other Oral Structures." The outstanding group of essayists includes: Dr. S. N. Bhaskar, Dr. Henry Goldman, Dr. Maynard K. Hine, Dr. Harry Sicher.

Further details and application form may be obtained by writing to Virginia B. Manella, Secretary, 1700 Mesa Rd., Colorado Springs, Colorado.

To the Editor . . . .

I want to take this opportunity to pay tribute to the memory of Arthur Hastings Merritt, whose death February 9 has saddened his many friends. Others who have worked more intimately with him than I in recent years, have already told the story of what he has meant to periodontology, the American Academy of Periodontology and its Journal in the July 1959 issue of THE JOURNAL OF PERIODONTOLOGY.

I would like to go back to my early years in dentistry and my association, both personally and in professional work, with this sterling man. In the years 1906-7, when in general practice in Binghamton, N.Y. I became interested in the general subject of dental hygiene as a factor in the health picture, especially the prevailing (at that time) neglect of children's teeth. This led to contact with Arthur who had the same basic interest. He became one of a group of New York City dentists who supported me in a petition to the New York State Dental Society to create a Dental Hygiene Council for New York State. Arthur, in addition to his other activities, worked very closely with me on that project.

I had only just begun at that time to be interested in periodontal disease ("pyorrhea" then) and I found that he was in about the same situation in that regard. While he was not one of the group that founded the Academy of Periodontology, he became a member soon after its founding. I might add that while there were some dentists who already had gained some recognition in that field but who failed to ally themselves with the Academy during its fledgling years, Arthur Merritt, recognizing even then the importance of periodontia as a branch of dentistry, not only joined the Academy but threw himself whole-heartedly into its activities.

Through that medium especially, Arthur and I have kept in close touch both personally and professionally through the years. Thus I have come to know better than most what Arthur Merritt has contributed to dentistry and to dental public health. I cannot close without paying tribute to him, not only as a close personal friend but as an outstanding dentist, and in addition a man of the highest character. Dentistry has too few Arthur Merritts.

John Oppie McCall
262 Fairhaven Mall, Jericho, N. Y.